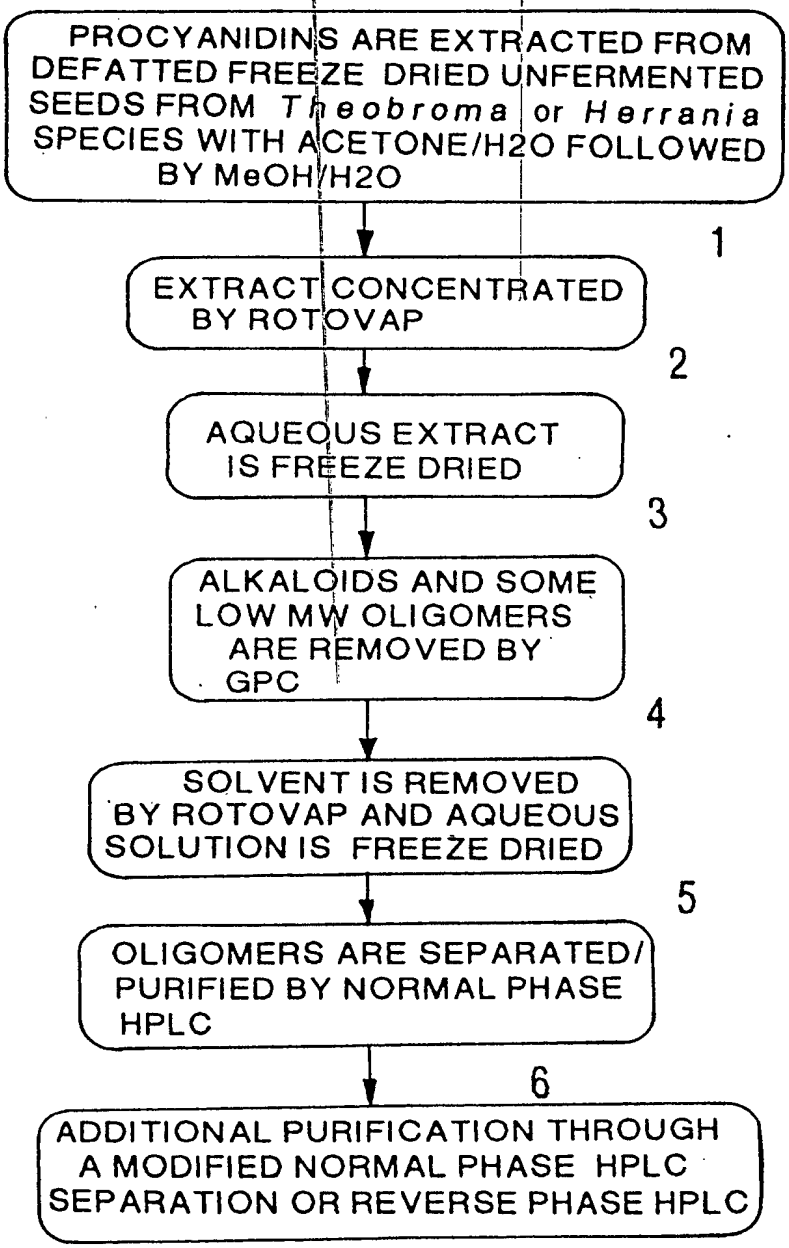
FIG.1

Summary of the current purification protocol



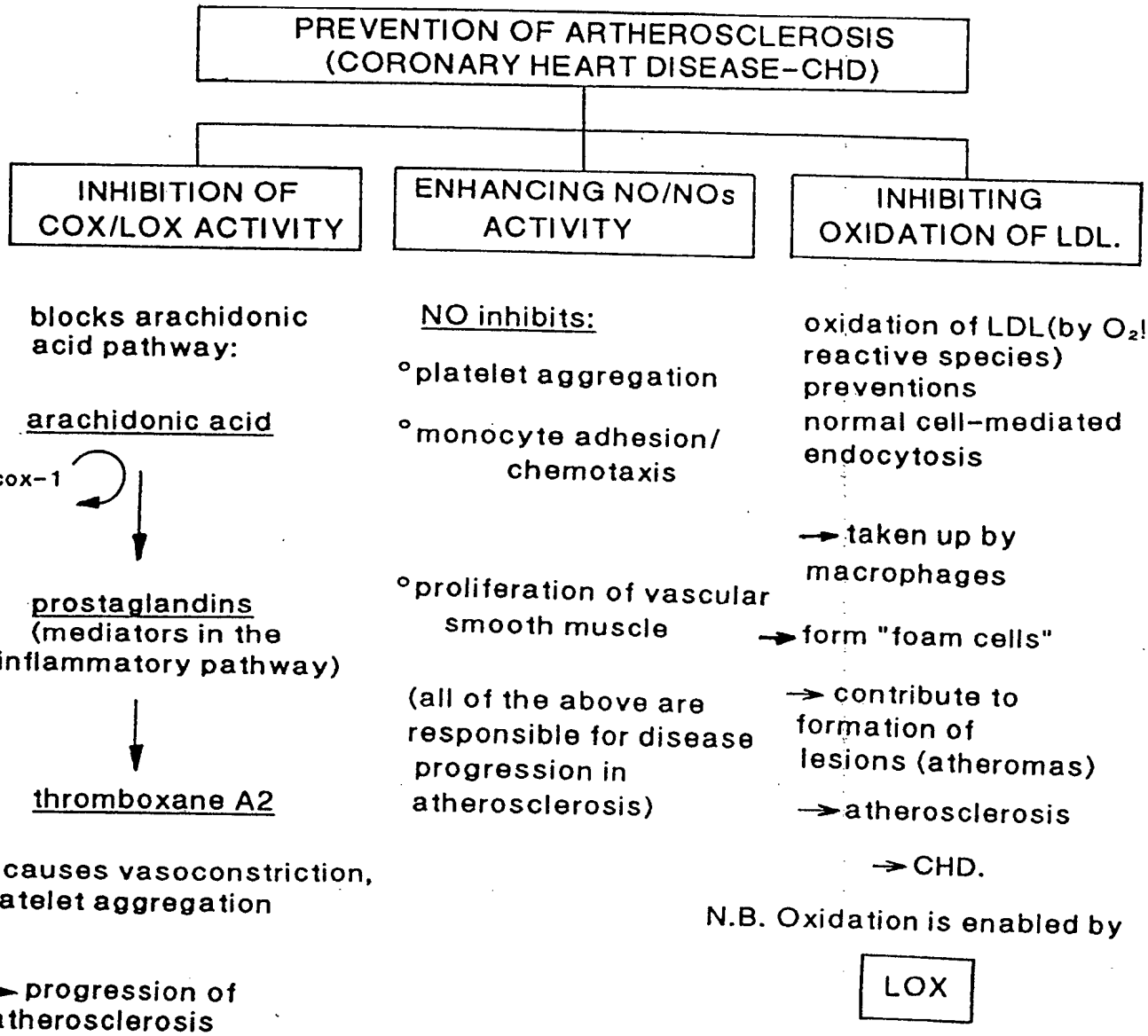
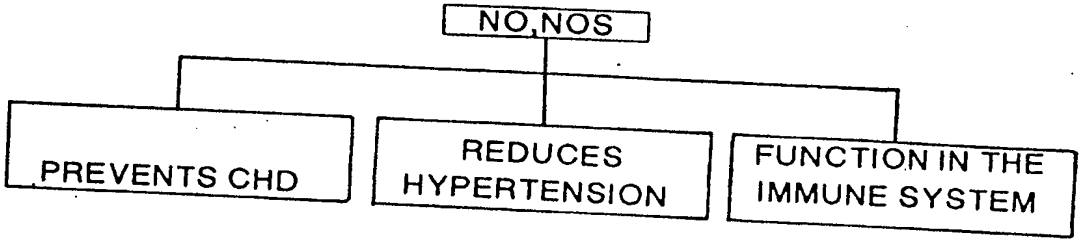


Chart showing the major contributing factors in the progression of CORONARY Heart Disease (CHD) and how the activity of cocoa procyanidins contributes to the prevention of the progression of the disease state

FIG.2 a

The cocoa procvanidins induce the activity of NOS and therefore the resulting production NO, thereby enhancing the health benefits mediated by the activity of nitric oxide (NO).



- inhibits platelet aggregation, monocyte adhesion, chemotaxis and vascular smooth muscle proliferation thereby causing vascular relaxation and preventing the disease progression of CHD.

By lowering blood pressure via the following mechanism:

- vascular endothelial cells release eNOS
- → result in production of NO
- → NO relaxes vascular smooth muscles, increasing vascular lumen diameter
- → lowers blood pressure
- → induces hypotension.

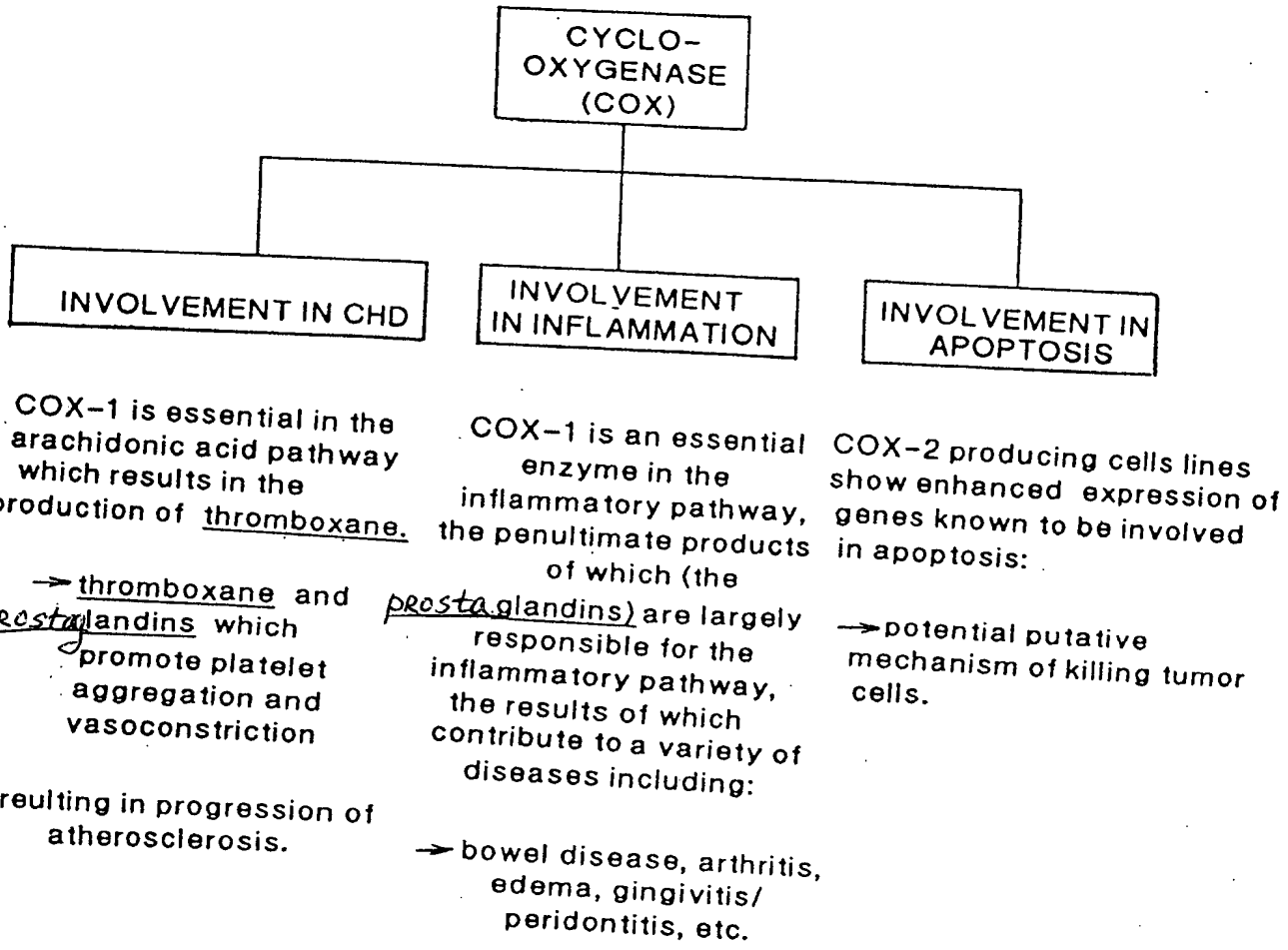
HYPERTENSION RESPONSIBLE FOR CARDIOVASCULAR DISEASES:

including:

stroke
heart attack
heart failure
kidney failur

- Macropages have a different NOS(iNOS)
- INOS gene transcription is controlled by cytokines
- iNOS activity results in macrophage NO production at sufficient concentrations to inhibit ribonuclease reductase
- → causes inhibition of DNA synthesis
- → potential mechanism of action in anti-tumor and anti-microbial function.

FIG.2b



The cocoa procyanidins inhibit the production of cyclo-oxygenase, thereby blocking the arachidonic acid pathway, which is responsible for the inflammatory response and the vasoconstrictive and platelet aggregating responses which contribute to the disease progression of CHD.

FIG.2c

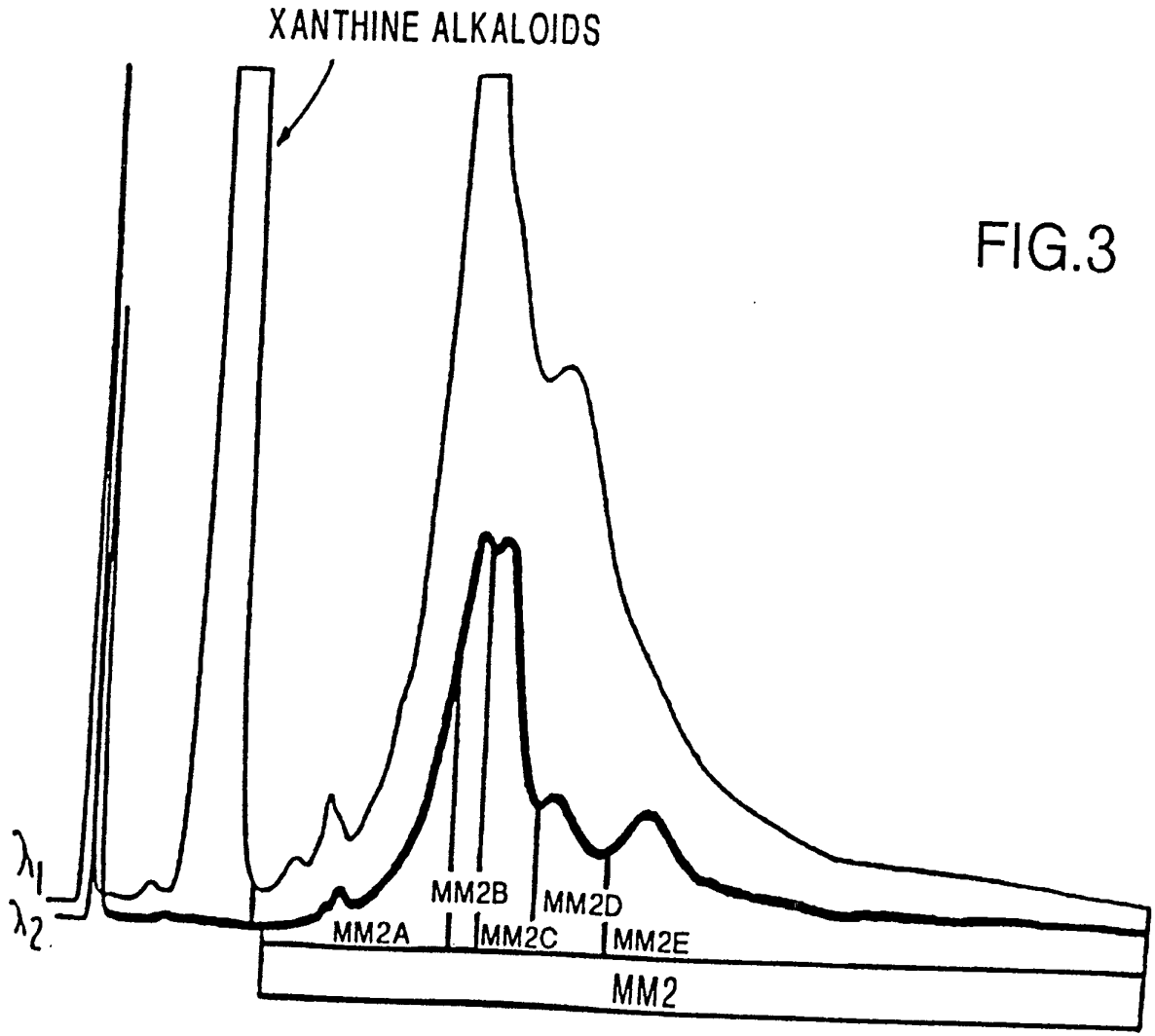


FIG.3

FIG.4

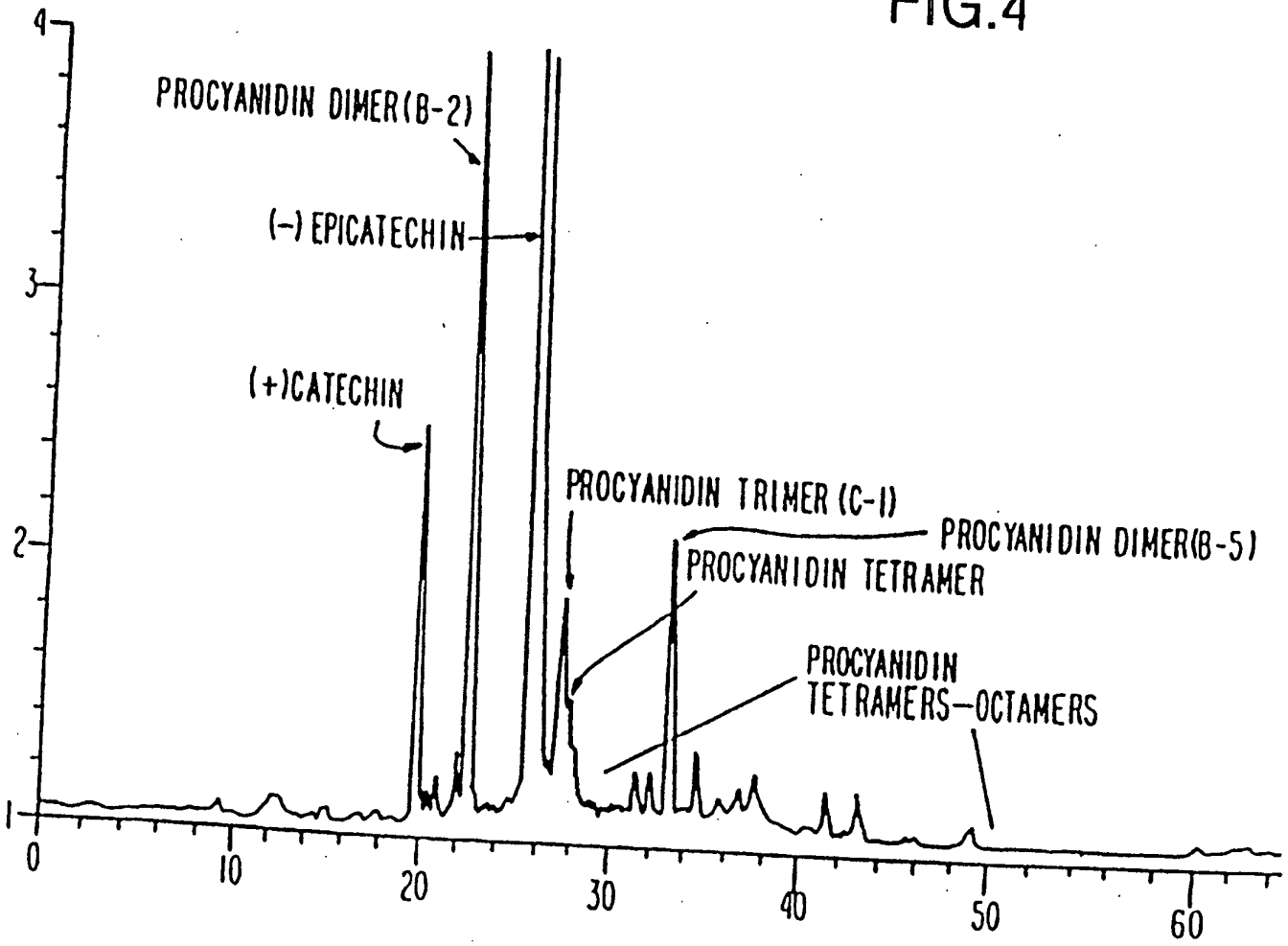

PROCYANIDIN DIMER(B-2)
(-) EPICATECHIN
(+)CATECHIN
PROCYANIDIN TRIMER (C-1)
PROCYANIDIN DIMER(B-5)
PROCYANIDIN TETRAMER
PROCYANIDIN
TETRAMERS-OCTAMERS
4
3
2
1
0
10
20
30
40
50
60

FIG.5

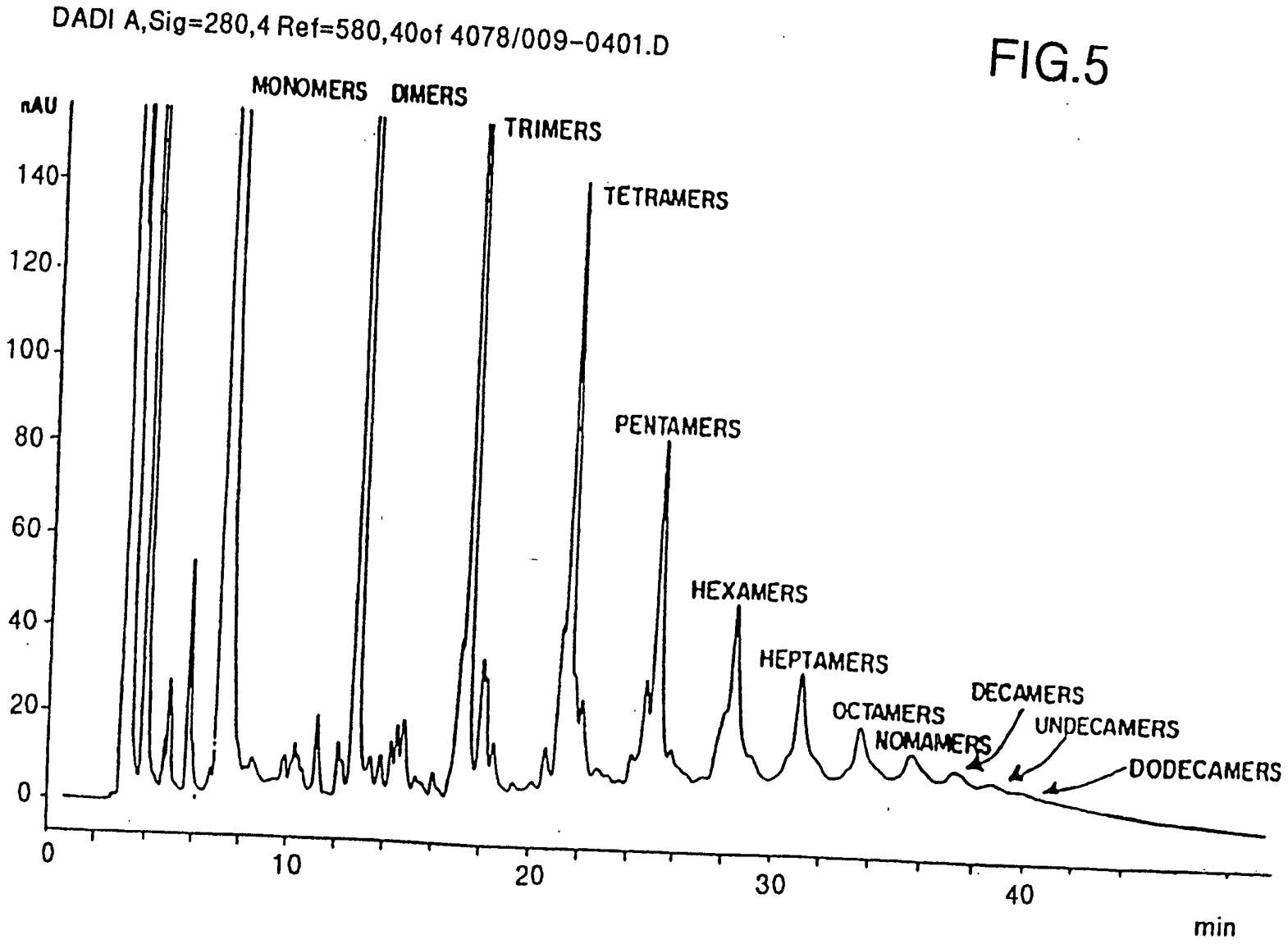

DADI A,Sig=280,4 Ref=580,40of 4078/009-0401.D
mAU
140
120
100
80
60
40
20
0
MONOMERS
DIMERS
TRIMERS
TETRAMERS
PENTAMERS
HEXAMERS
HEPTAMERS
OCTAMERS
NOMAMERS
DECAMERS
UNDECAMERS
DODECAMERS
0
10
20
30
40
min

FIG.6

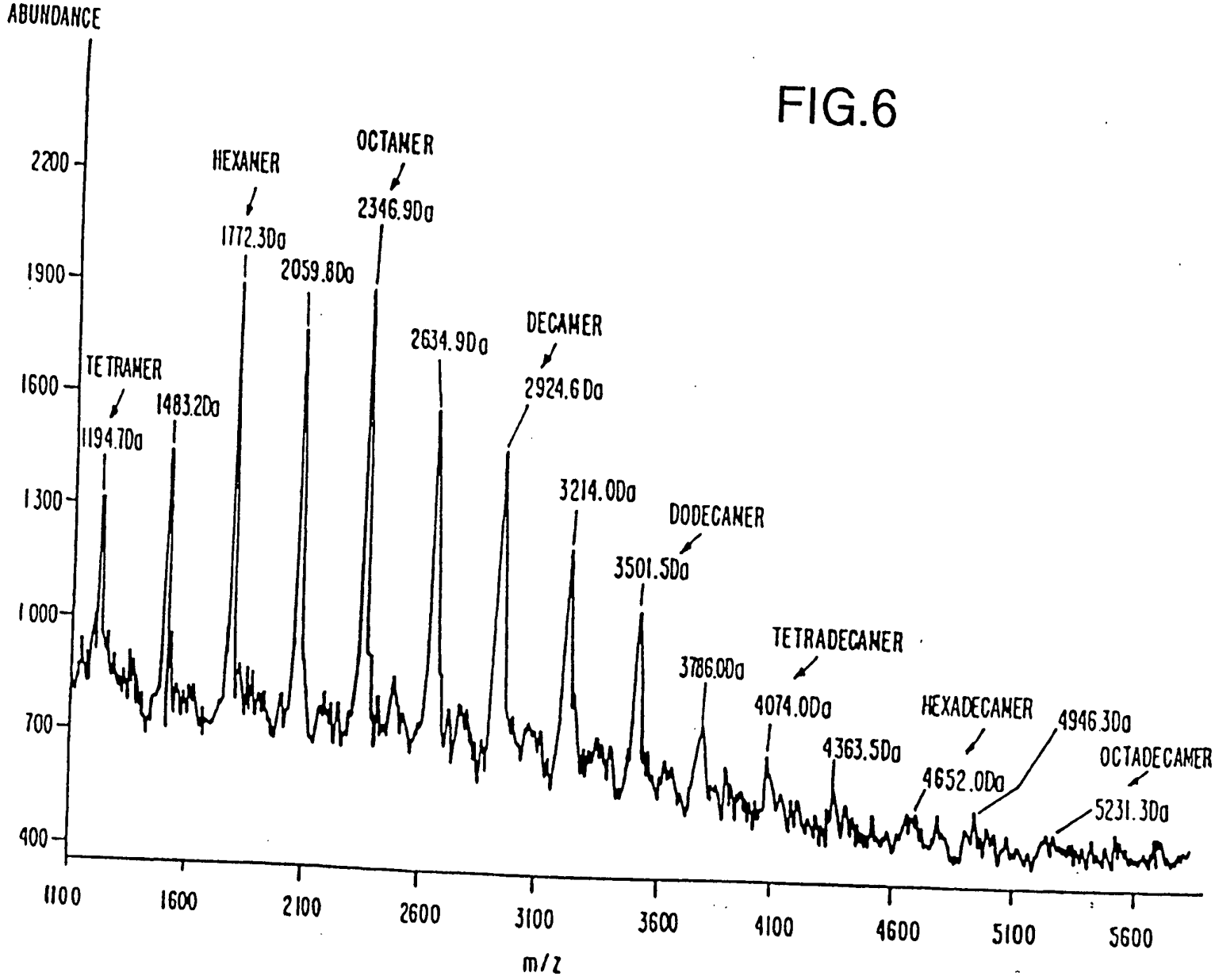

ABUNDANCE
2200
1900
1600
1300
1000
700
400
TETRAMER
1194.7Da
1483.2Da
HEXAMER
1772.3Da
2059.8Da
OCTAMER
2346.9Da
2634.9Da
DECAMER
2924.6Da
3214.0Da
DODECAMER
3501.5Da
3786.0Da
TETRADECAMER
4074.0Da
4363.5Da
HEXADECAMER
4652.0Da
4946.3Da
OCTADECAMER
5231.3Da
1100
1600
2100
2600
3100
3600
4100
4600
5100
5600
m/z

FIG.7

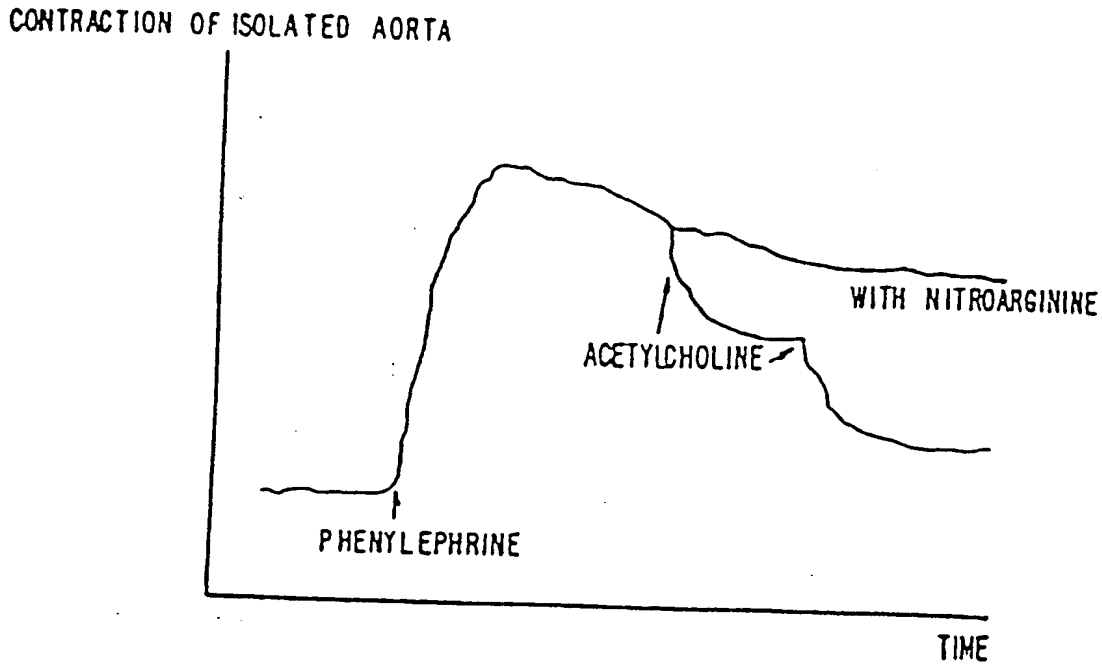
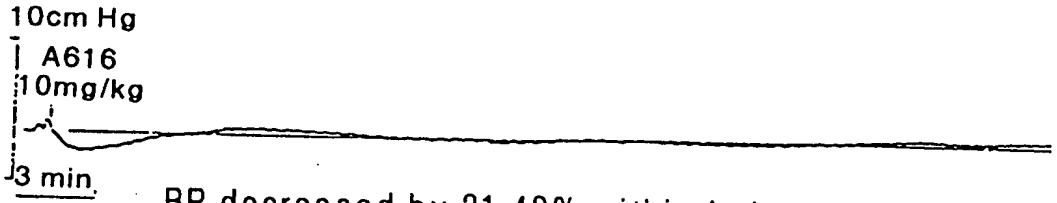

CONTRACTION OF ISOLATED AORTA
WITH NITROARGININE
ACETYLCHOLINE
PHENYLEPHRINE
TIME

FIG.8A

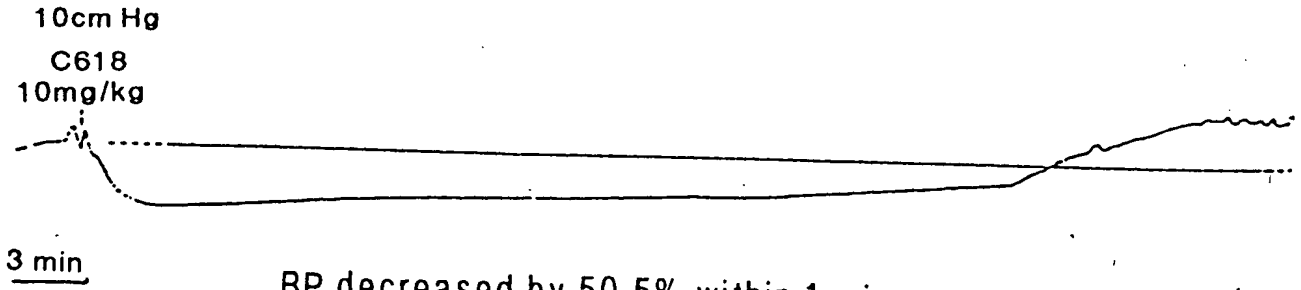
EFFECT OF COCOA PROCYANIDIN FRACTION A ON BLOOD PRESSURE



BP decreased by 21.43% within 1min
BP back to normal value after 1.5 min

FIG.8B

EFFECT OF COCOA PROCYANIDIN FRACTION C ON BLOOD PRESSURE



BP decreased by 50.5% within 1 min
BP back to normal value after 5 min

EFFECT OF COCOA PROCYANIDIN FRACTIONS ON ARTERIAL BLOOD PRESSURE IN ANESTHESIZED GUINEA PIGS

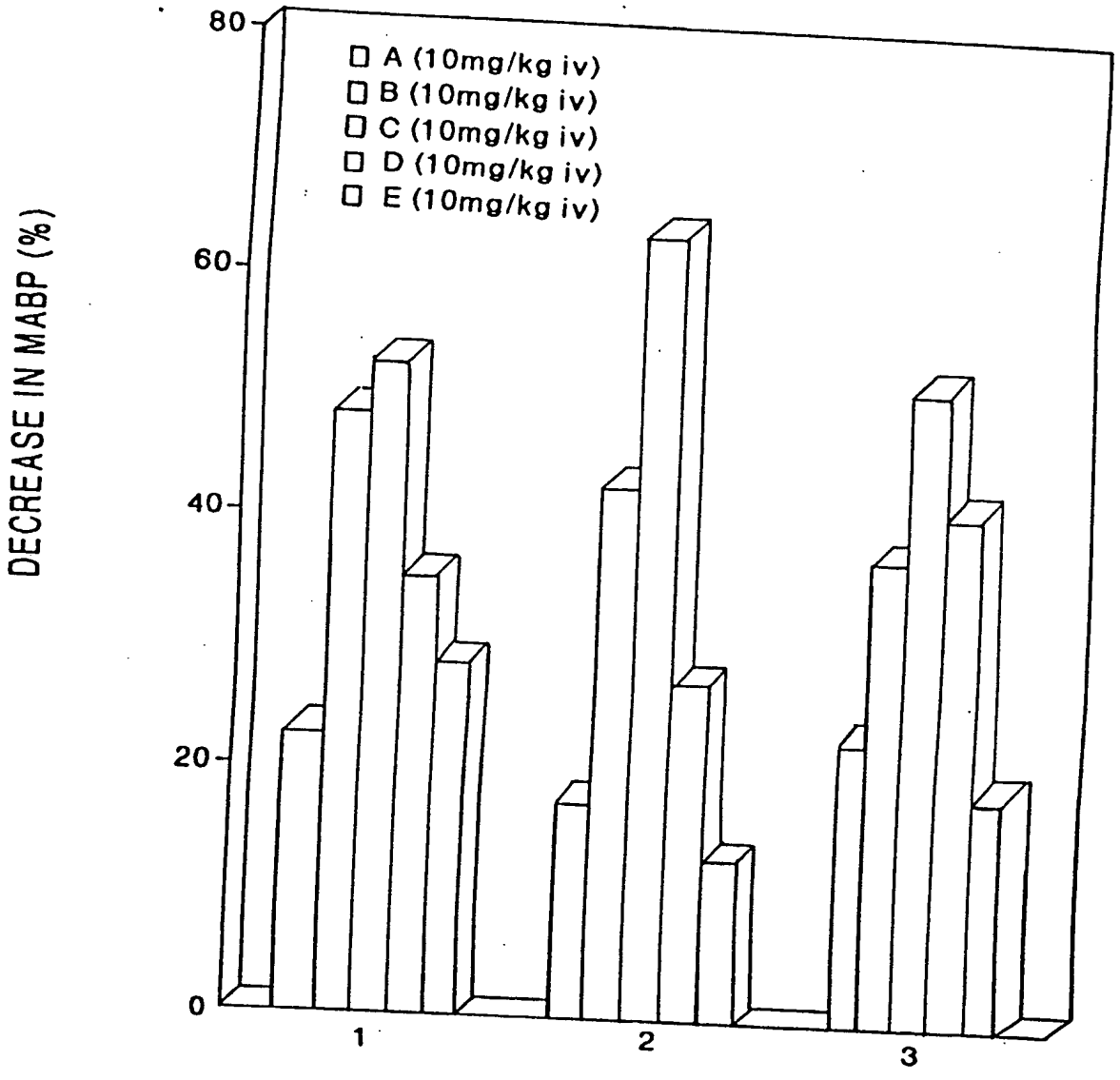


FIG.9

EFFECT OF L-NMMA ON THE ALTERATIONS OF ARTERIAL BLOOD PRESSURE IN ANESTHESIZED GUINEA PIGS INDUCED BY COCOA PROCYANIDIN FRACTION C

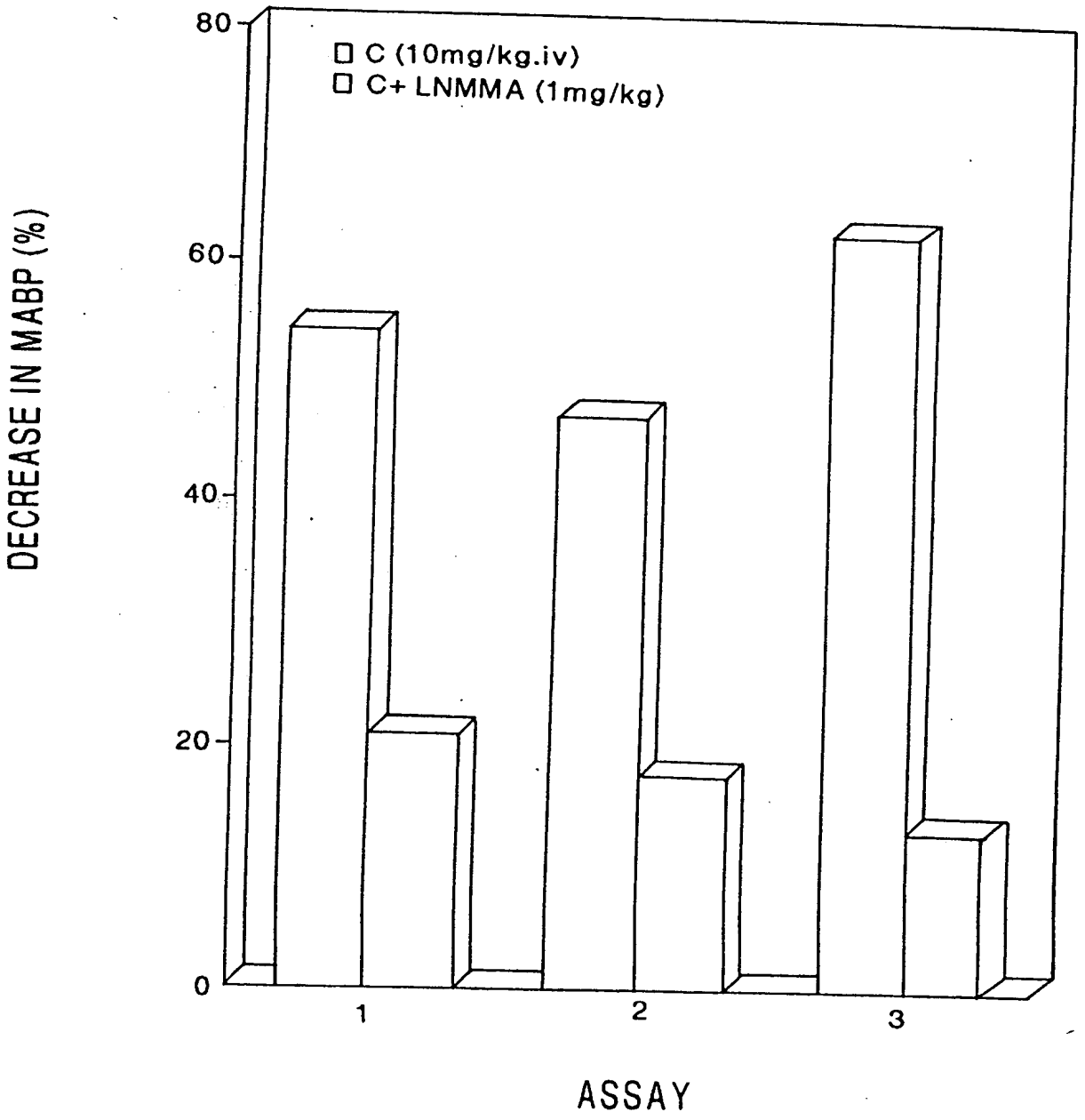


FIG.10

EFFECT OF BRADYKININ ON NO PRODUCTION BY HUVEC

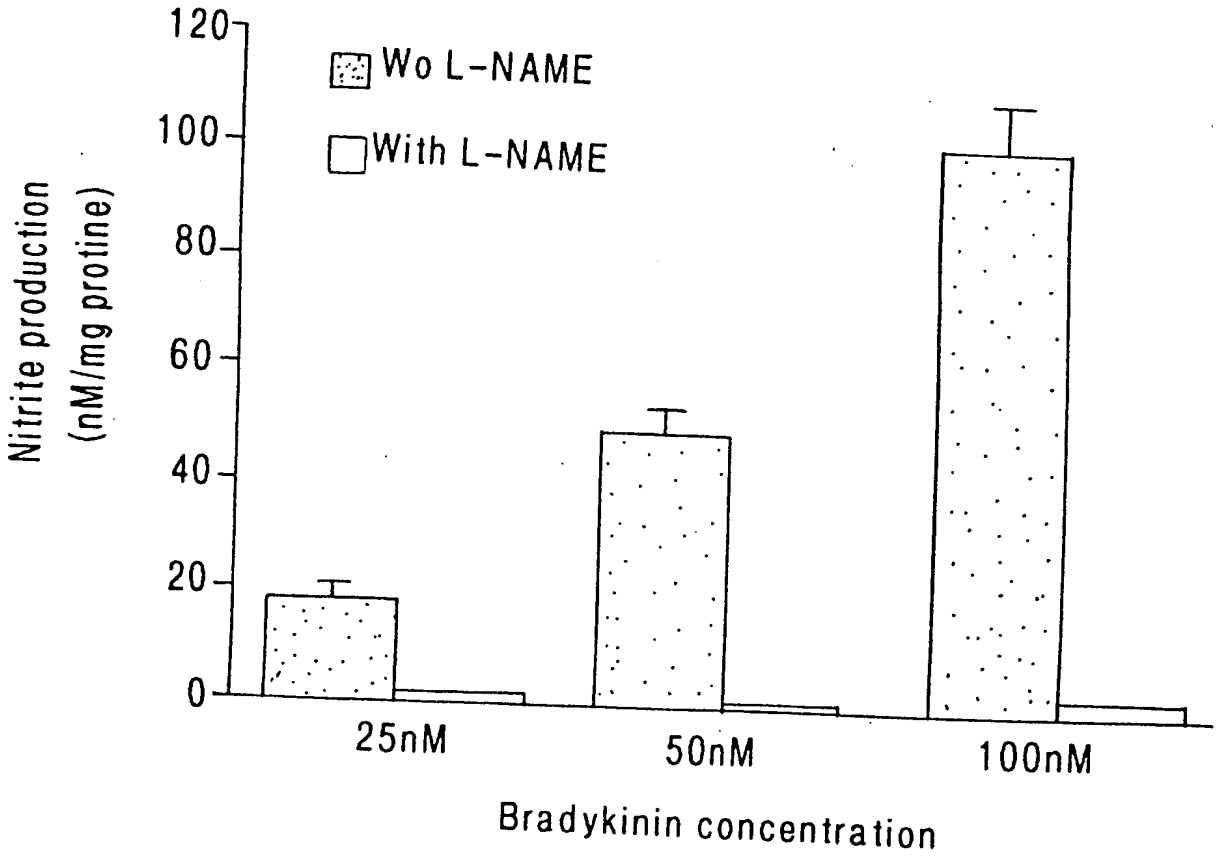


FIG.11

EFFECT OF COCOA PROCYANIDIN FRACTIONS ON NO PRODUCTION BY HUVEC

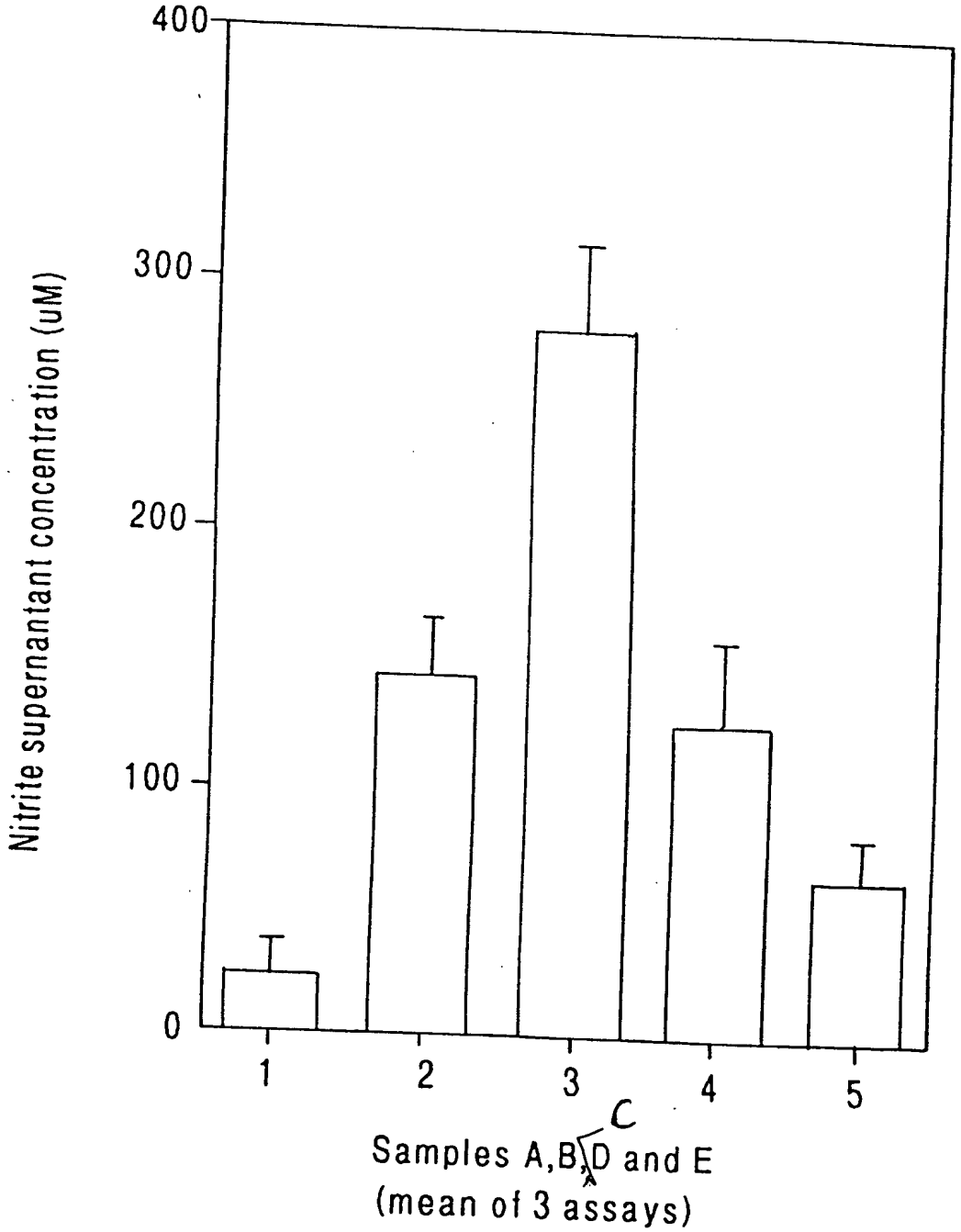


FIG.12

Figure A:Effect of Cocoa Procyanidin Fractions on Macrophage NO Production

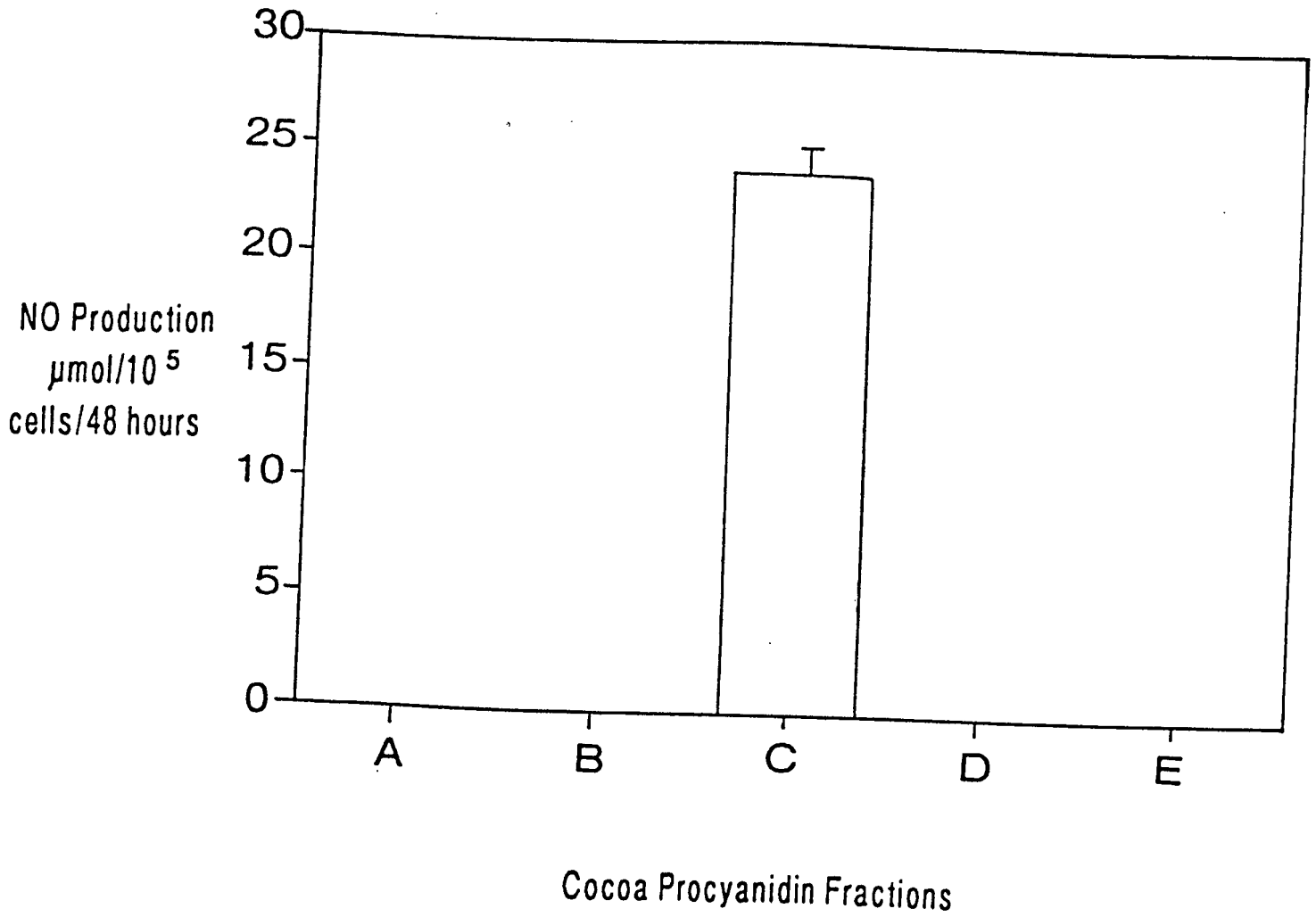


FIG.13

Figure B:Effect of Cocoa Procyanidin Fractions on LPS Induced and γ-Interferon Primed Macrophages

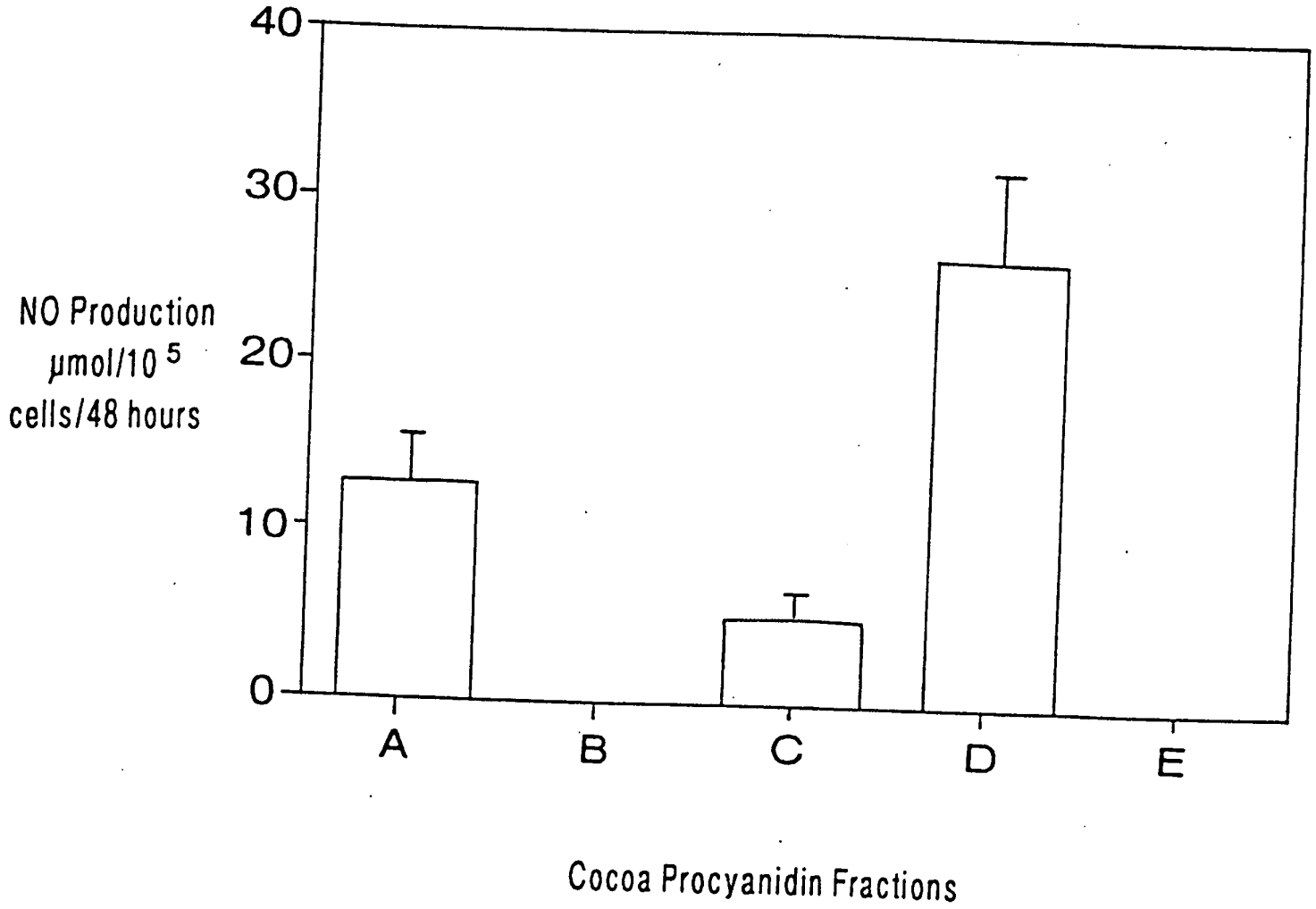


FIG.14

FIG.15A

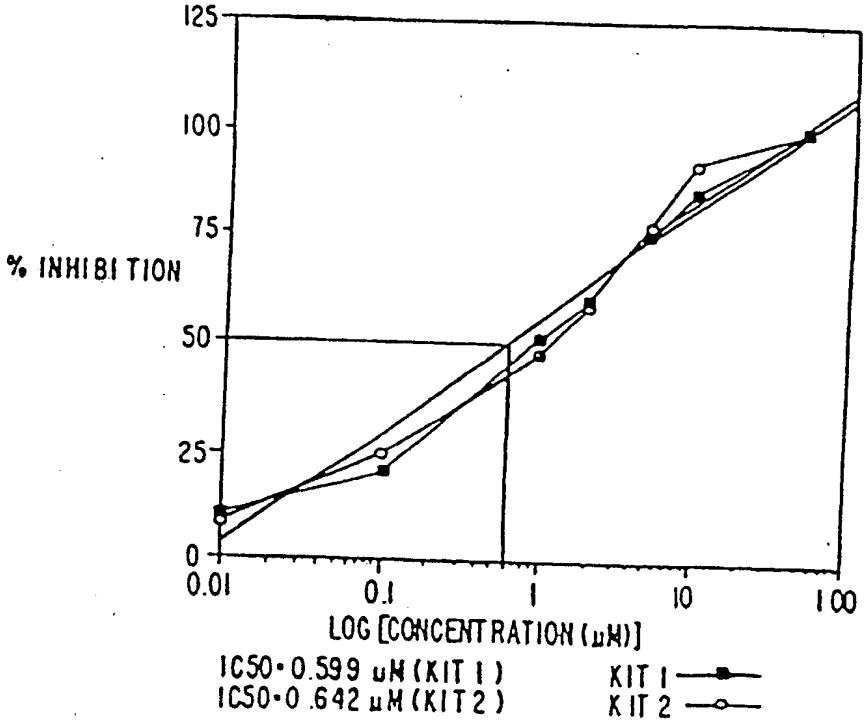


FIG.15B

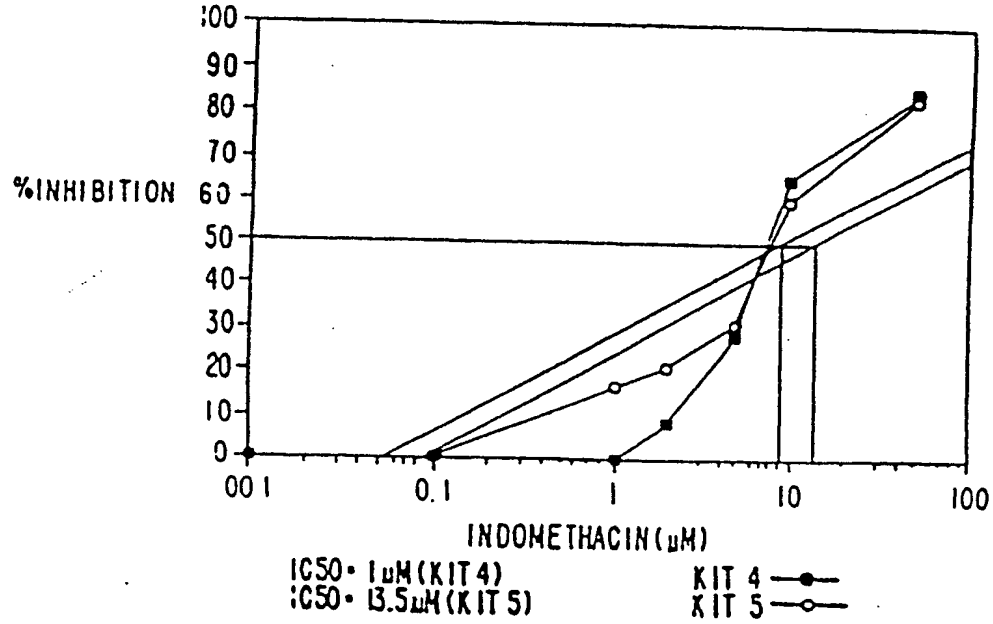
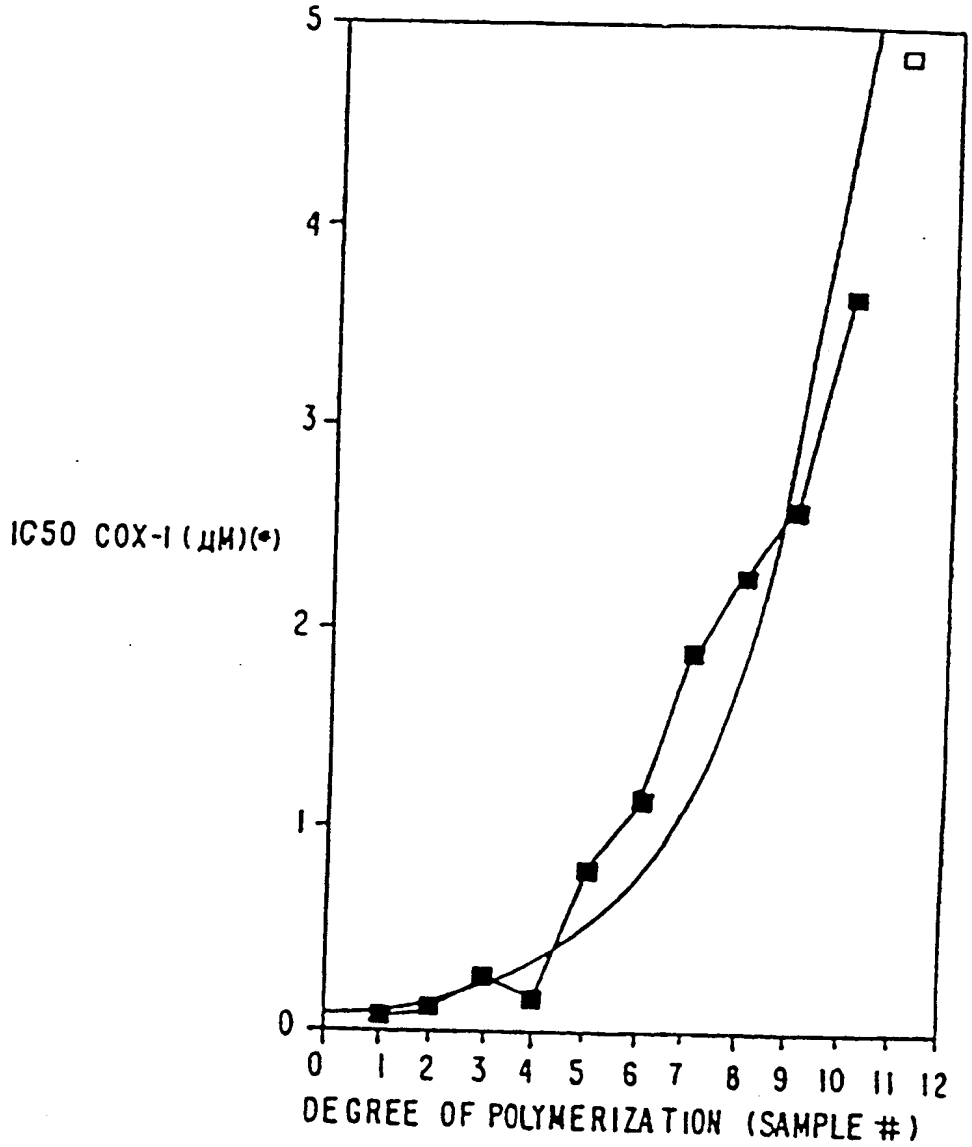
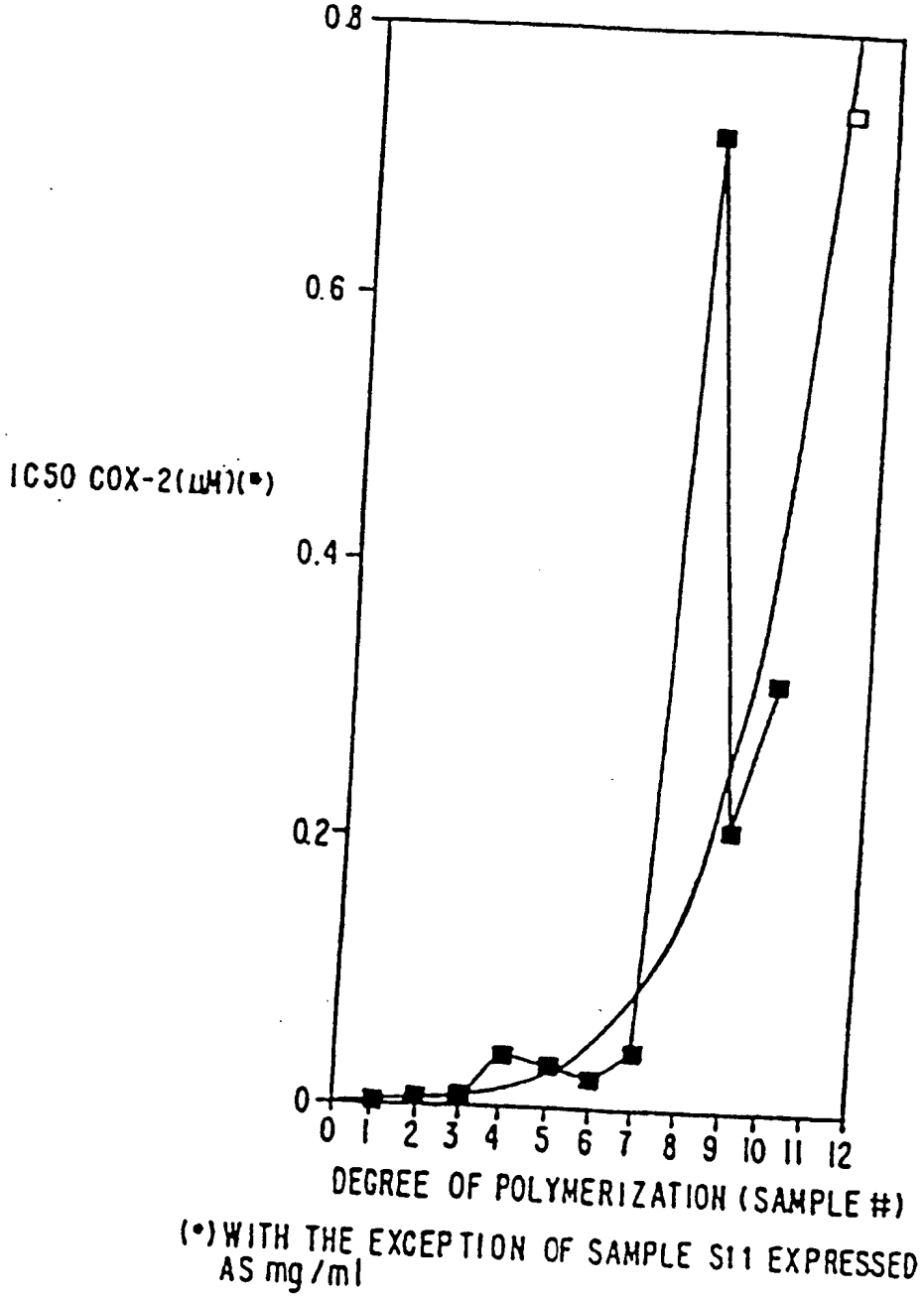


FIG.16A



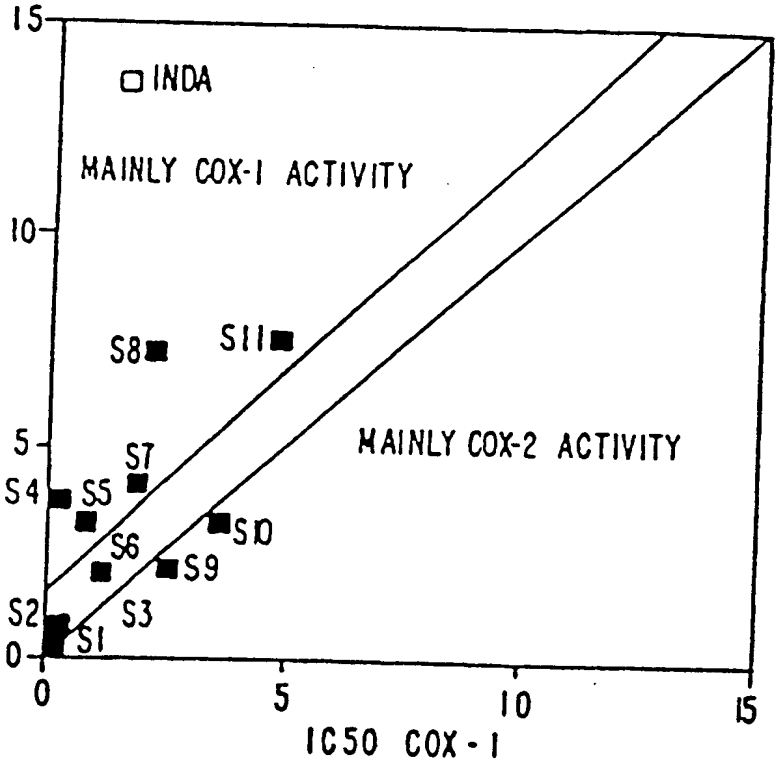
(*) WITH THE EXCEPTION OF SAMPLE SII EXPRESSED AS mg/ml

FIG.16B



(*) WITH THE EXCEPTION OF SAMPLE S11 EXPRESSED AS mg/ml

FIG.17



(*) WITH THE EXEPTION OF SAMPLE S11

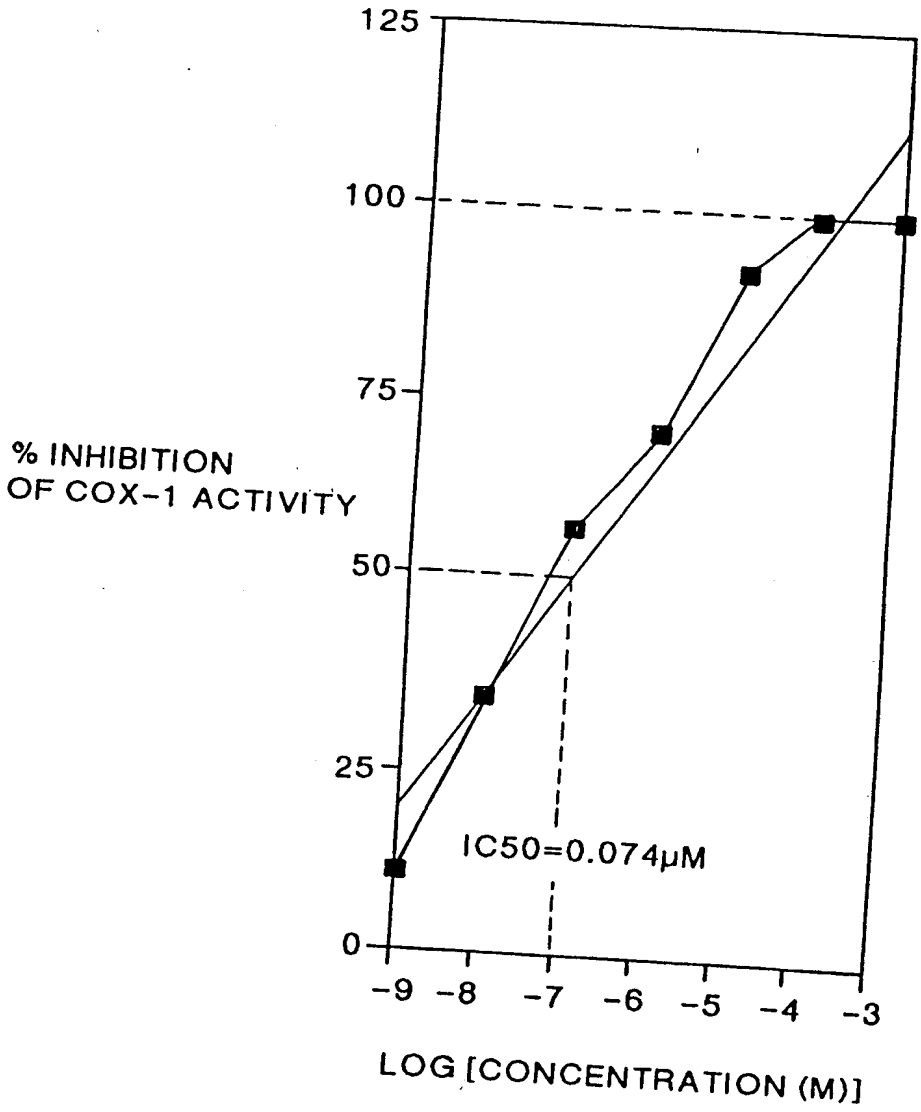

125
100
75
50
25
0
% INHIBITION
OF COX-1 ACTIVITY
IC50=0.074μM
-9 -8 -7 -6 -5 -4 -3
LOG [CONCENTRATION (M)]

FIG.18A

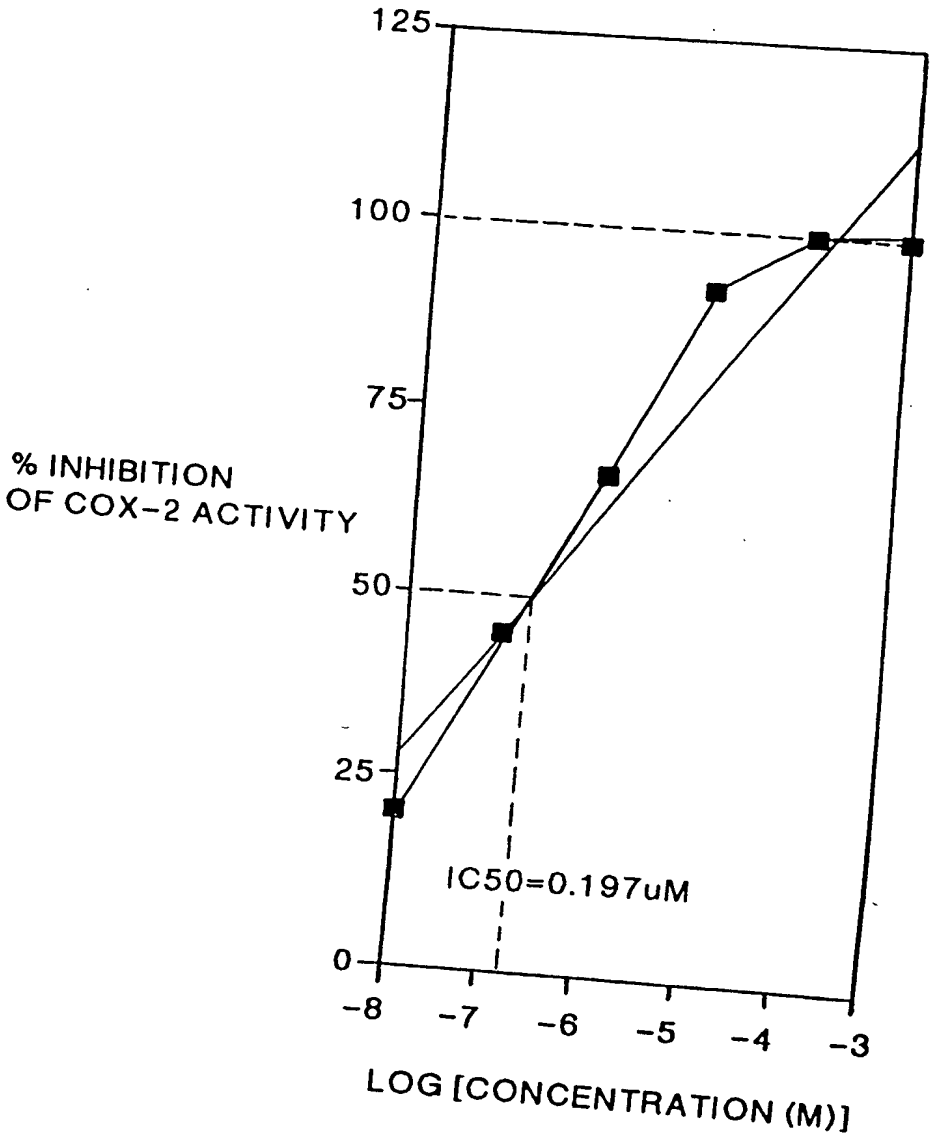

125
100
75
50
25
0
% INHIBITION
OF COX-2 ACTIVITY
IC50=0.197uM
-8
-7
-6
-5
-4
-3
LOG [CONCENTRATION (M)]

FIG.18B

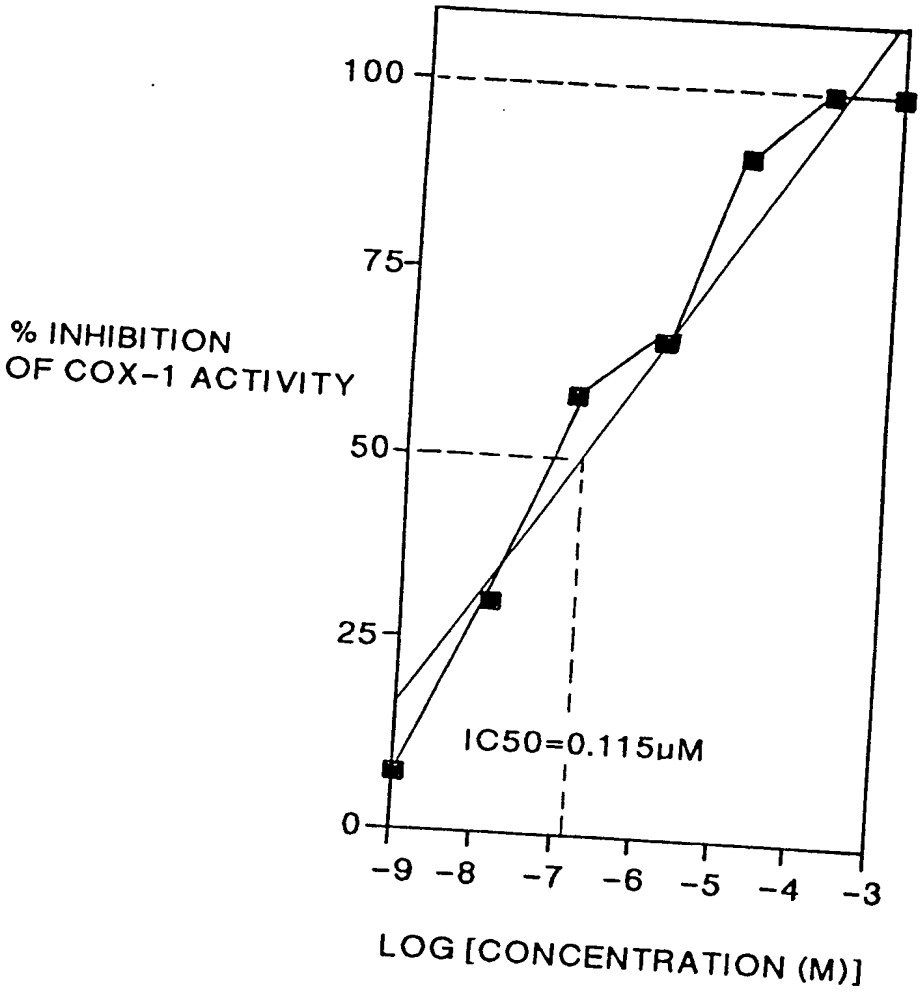

100
75
50
25
0
% INHIBITION
OF COX-1 ACTIVITY
IC50=0.115μM
-9
-8
-7
-6
-5
-4
-3
LOG [CONCENTRATION (M)]

FIG.18C

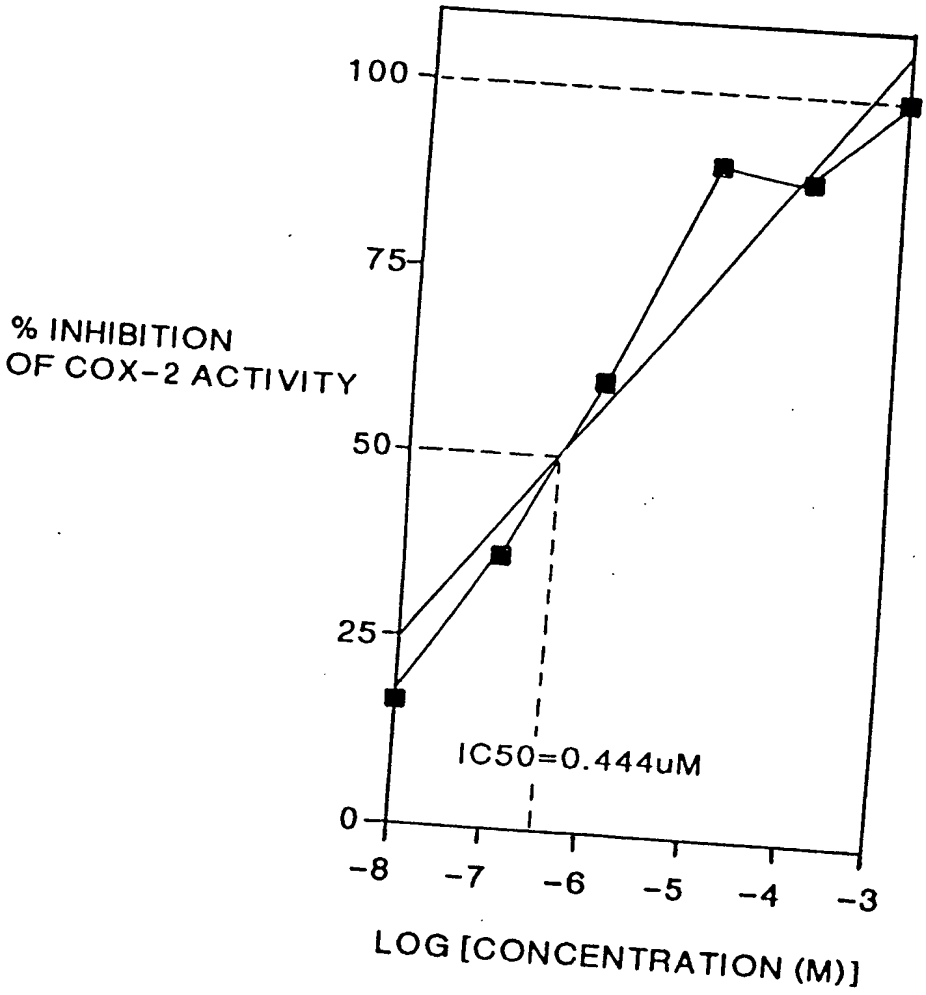

100
75
50
25
0
% INHIBITION
OF COX-2 ACTIVITY
IC50=0.444uM
-8
-7
-6
-5
-4
-3
LOG [CONCENTRATION (M)]

FIG.18D

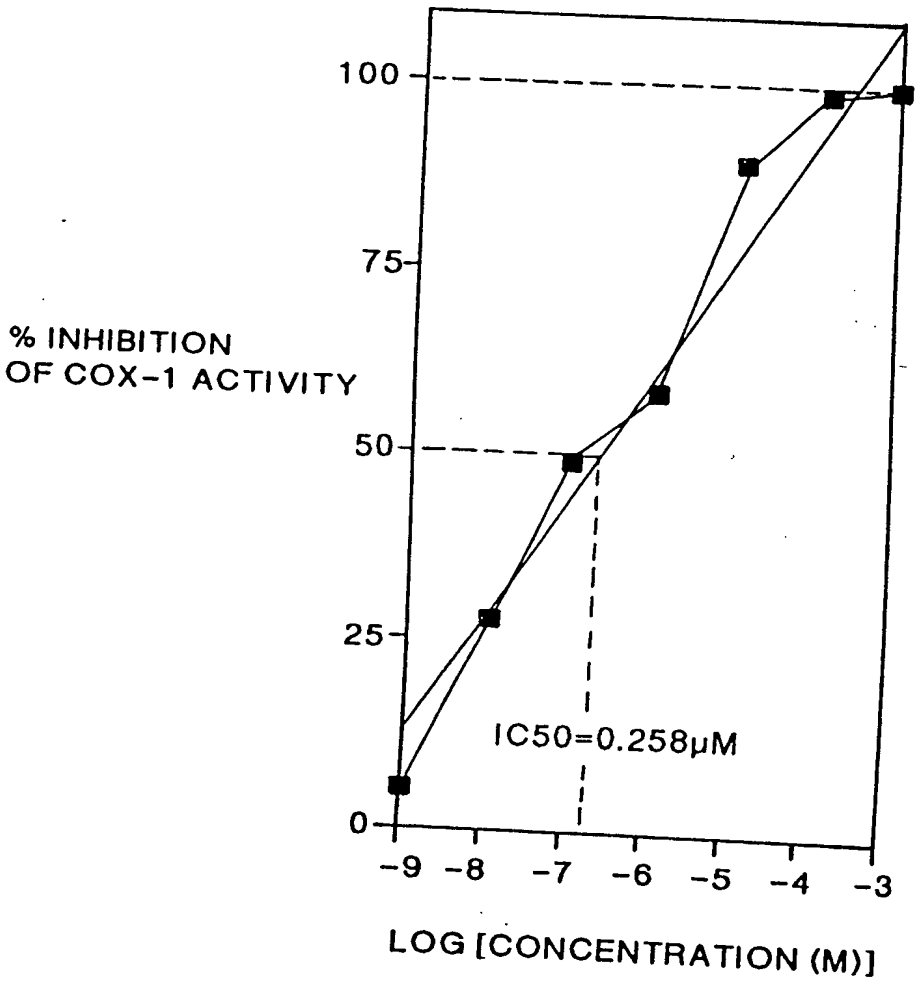

100
75
50
25
0
% INHIBITION
OF COX-1 ACTIVITY
IC50=0.258μM
-9
-8
-7
-6
-5
-4
-3
LOG [CONCENTRATION (M)]

FIG.18E

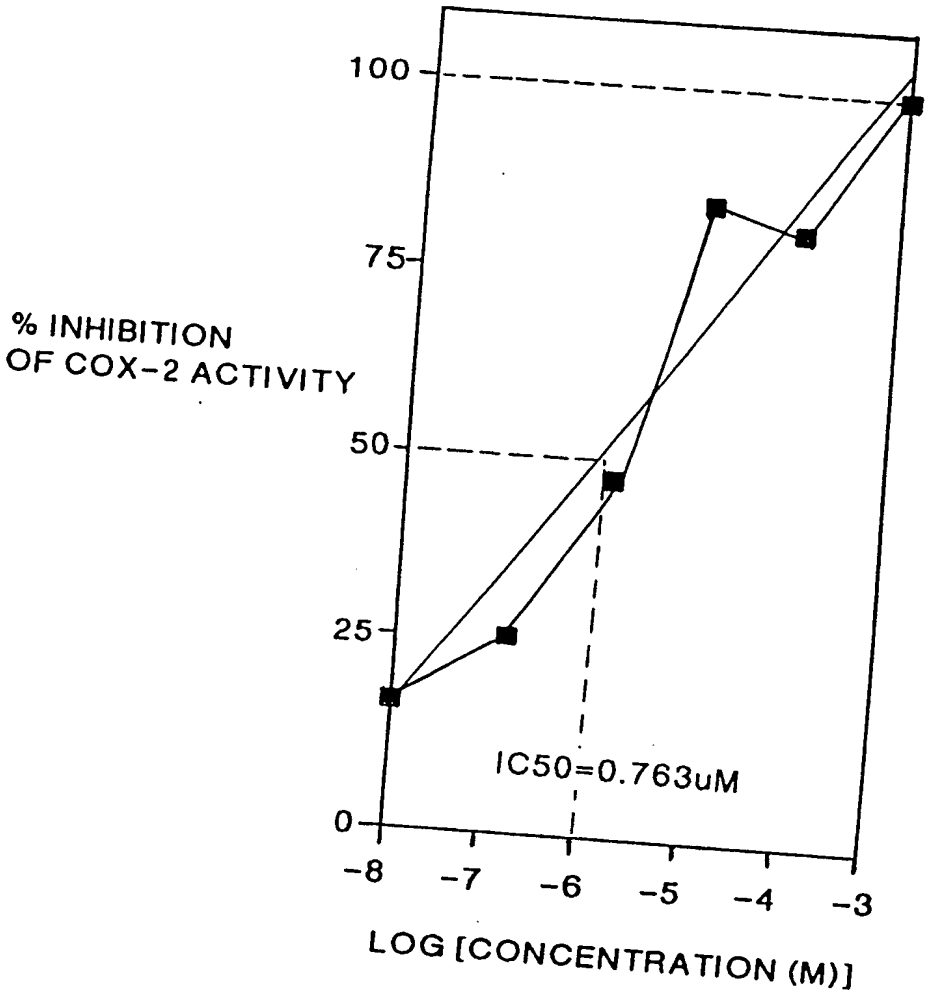

100
75
50
25
0
% INHIBITION
OF COX-2 ACTIVITY
IC50=0.763uM
-8
-7
-6
-5
-4
-3
LOG [CONCENTRATION (M)]

FIG.18F

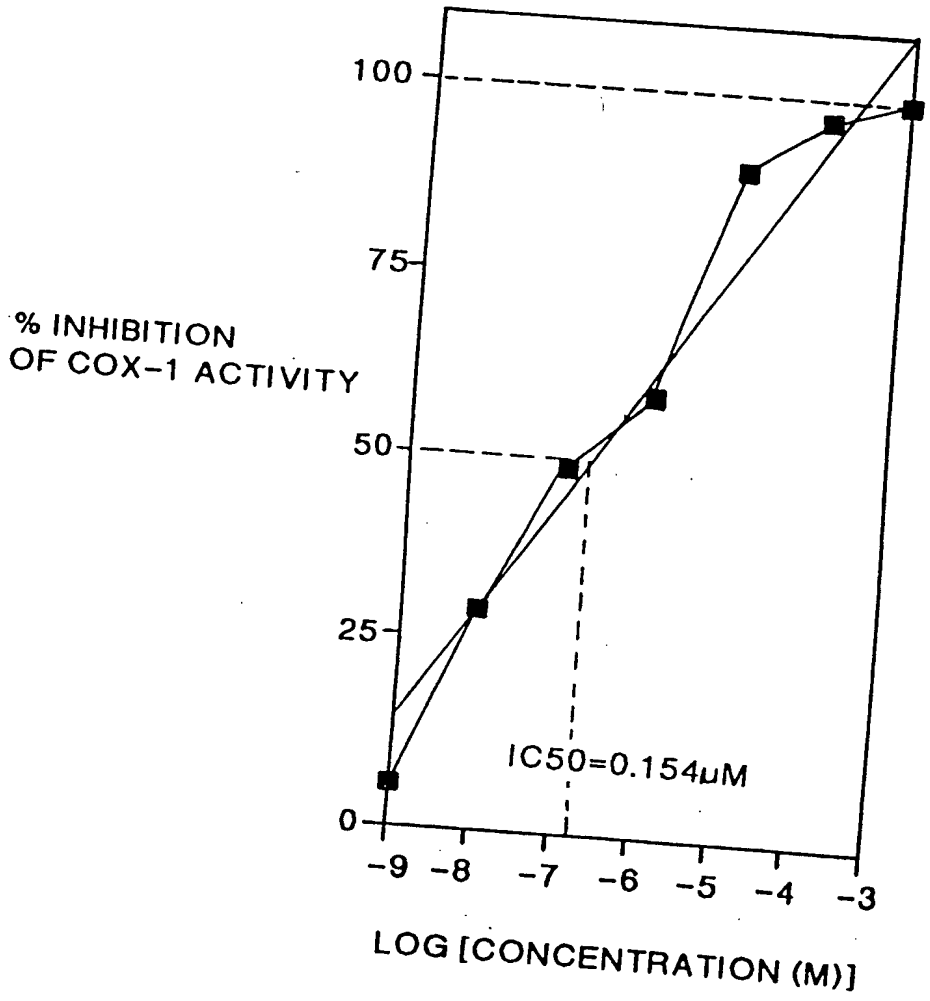

100
75
50
25
0
% INHIBITION
OF COX-1 ACTIVITY
IC50=0.154μM
-9
-8
-7
-6
-5
-4
-3
LOG [CONCENTRATION (M)]

FIG.18G

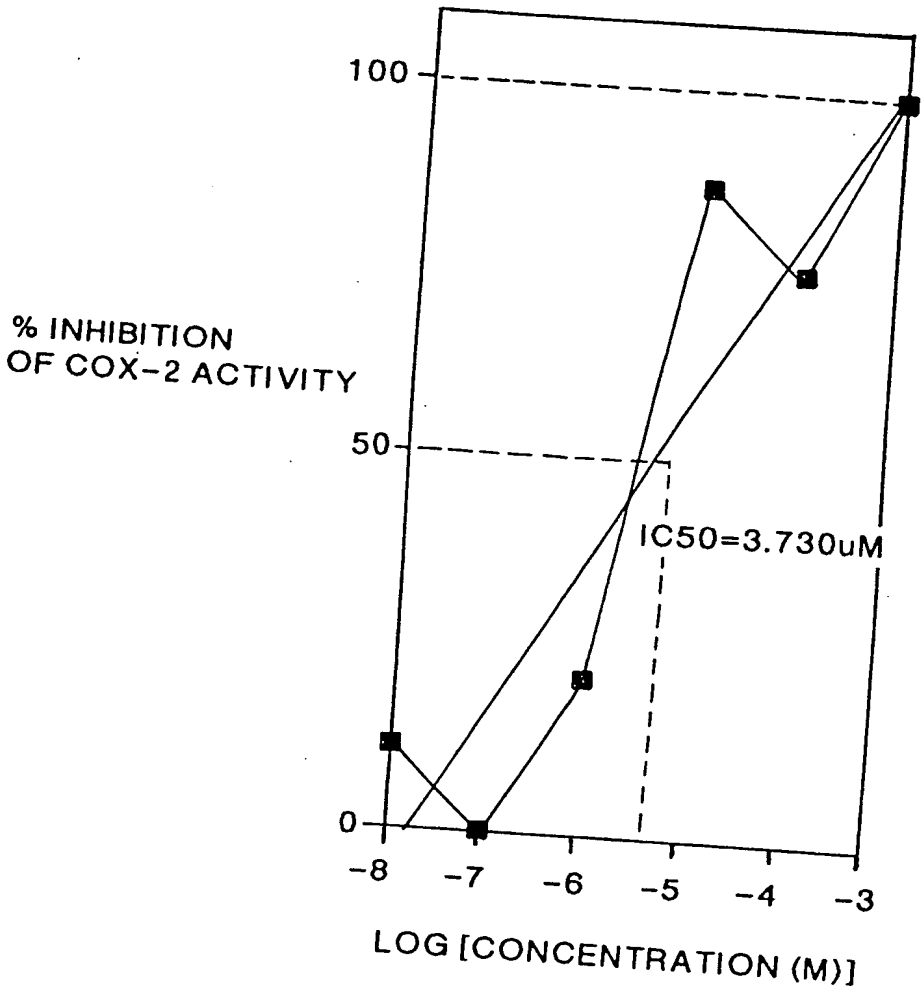

% INHIBITION
OF COX-2 ACTIVITY
100
50
0
IC50=3.730uM
-8
-7
-6
-5
-4
-3
LOG [CONCENTRATION (M)]

FIG.18H

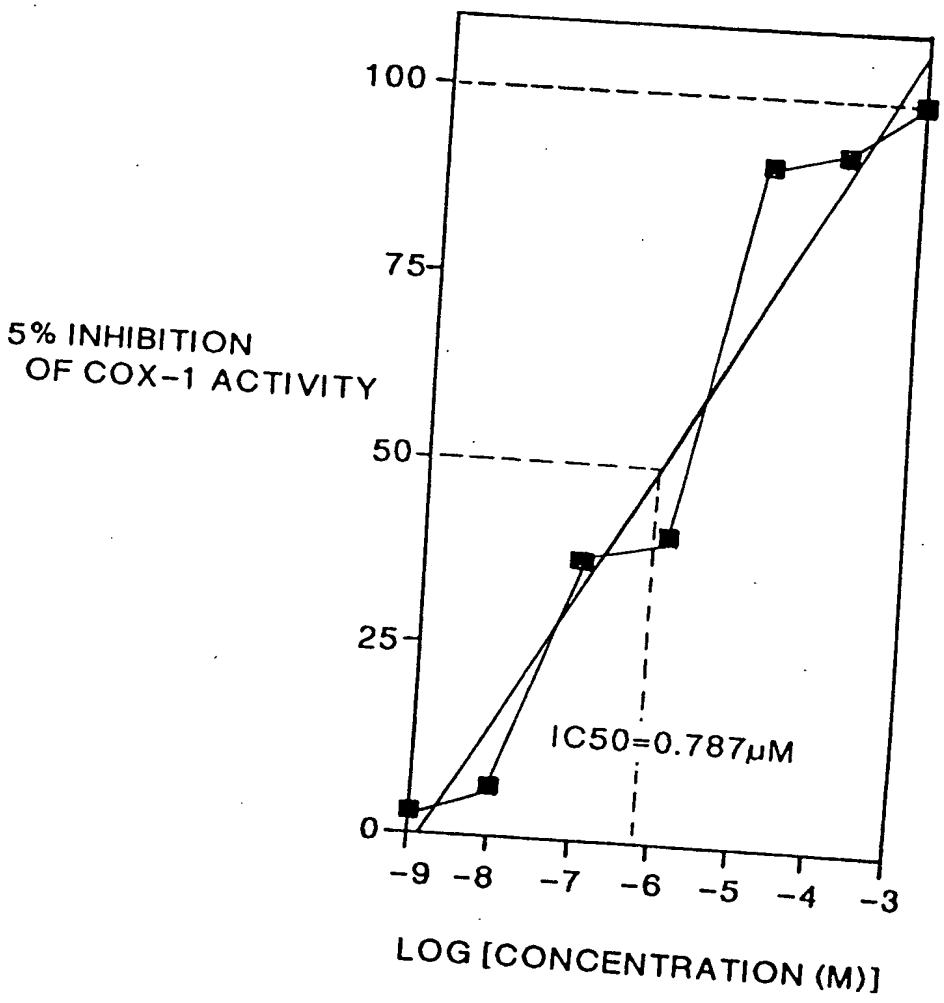

100
75
50
25
0
5% INHIBITION
OF COX-1 ACTIVITY
IC50=0.787μM
-9
-8
-7
-6
-5
-4
-3
LOG [CONCENTRATION (M)]

FIG.18 I

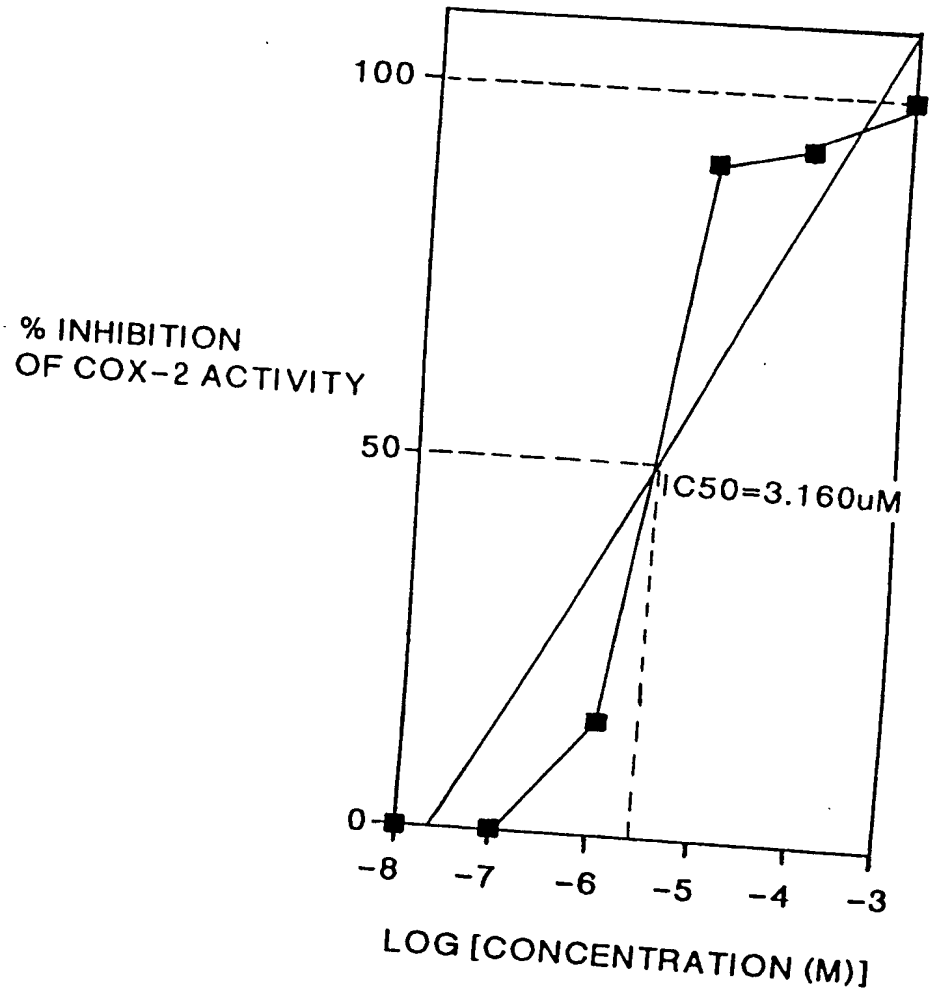

100
50
0
% INHIBITION
OF COX-2 ACTIVITY
IC50=3.160uM
-8
-7
-6
-5
-4
-3
LOG [CONCENTRATION (M)]

FIG.18J

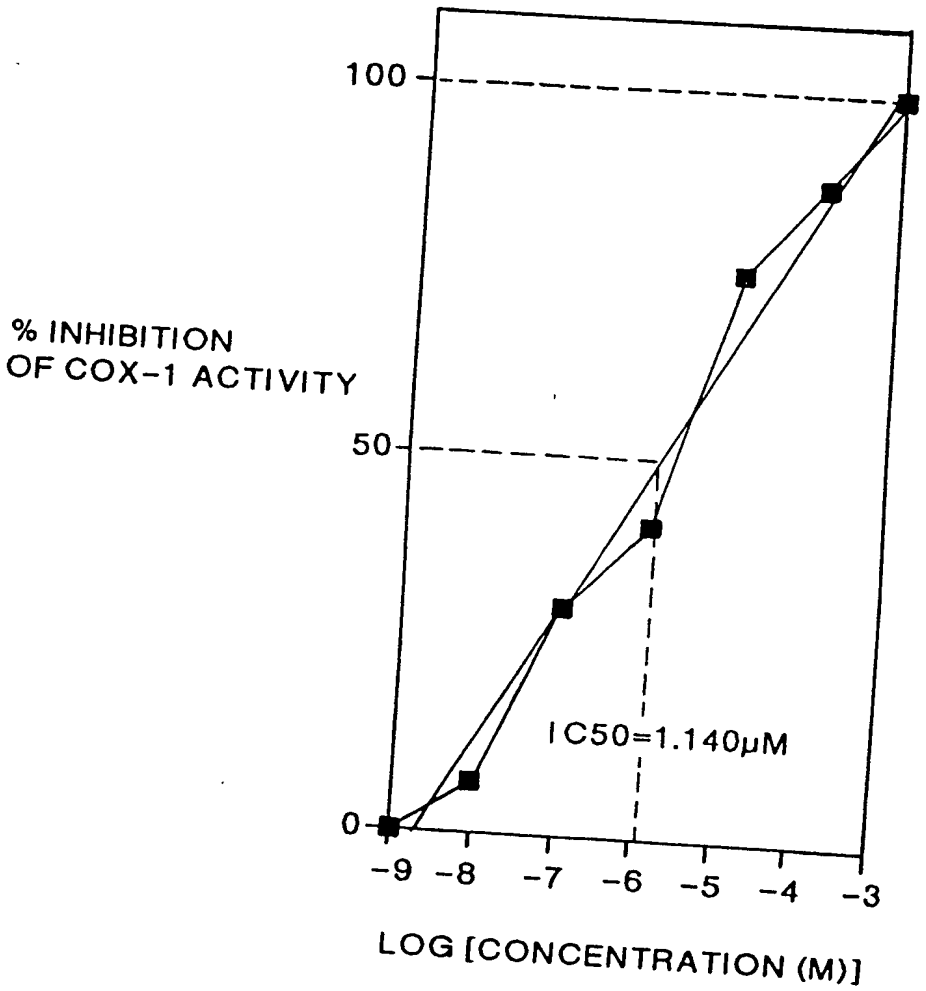

100
50
0
% INHIBITION
OF COX-1 ACTIVITY
IC50=1.140μM
-9
-8
-7
-6
-5
-4
-3
LOG [CONCENTRATION (M)]

FIG.18K

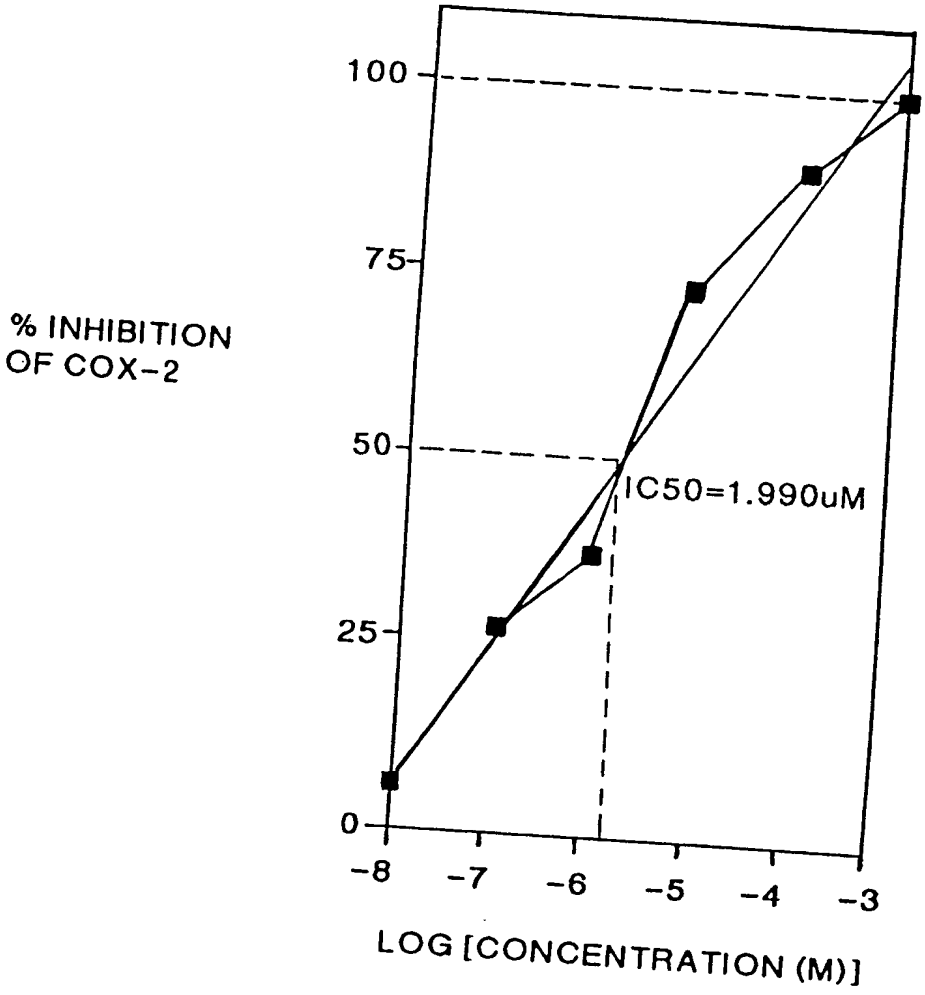

% INHIBITION
OF COX-2
100
75
50
25
0
IC50=1.990uM
-8
-7
-6
-5
-4
-3
LOG [CONCENTRATION (M)]

FIG.18L

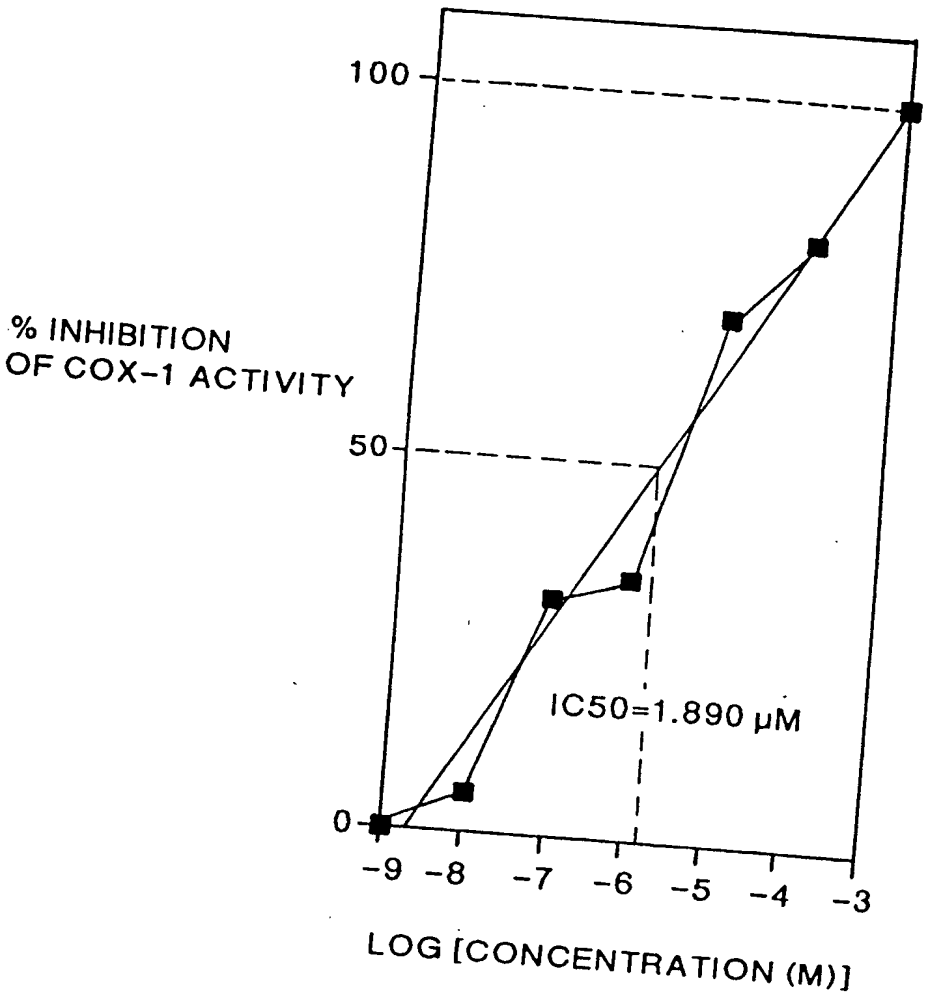

100
50
0
% INHIBITION
OF COX-1 ACTIVITY
IC50=1.890 μM
-9
-8
-7
-6
-5
-4
-3
LOG [CONCENTRATION (M)]

FIG.18M

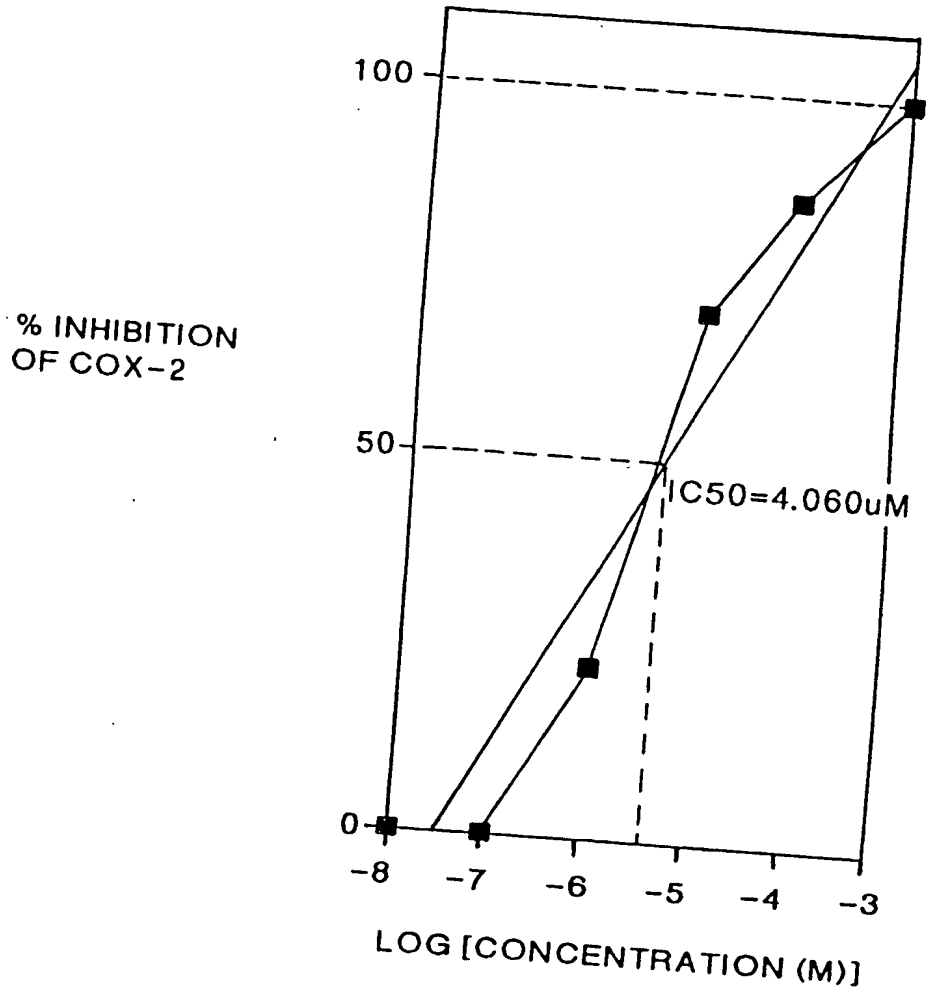

% INHIBITION OF COX-2
100
50
0
IC50=4.060uM
-8
-7
-6
-5
-4
-3
LOG [CONCENTRATION (M)]

FIG.18N

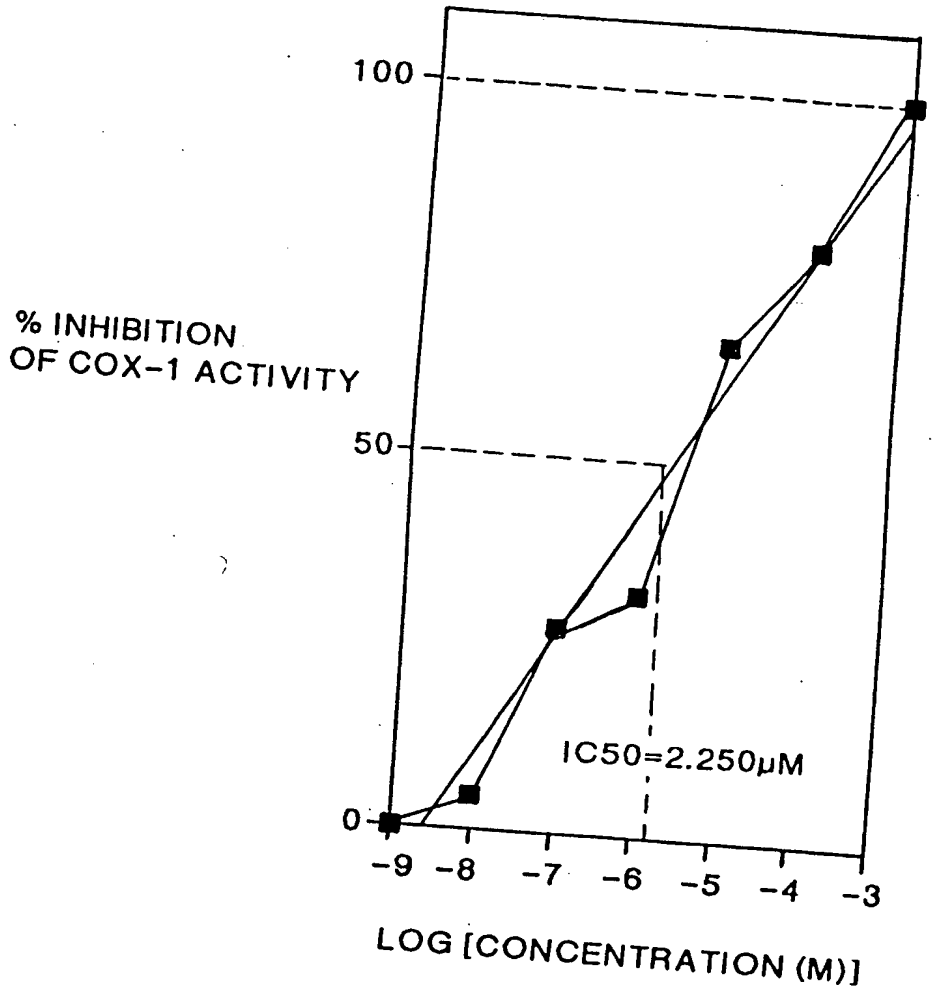

100
50
0
% INHIBITION
OF COX-1 ACTIVITY
IC50=2.250μM
-9
-8
-7
-6
-5
-4
-3
LOG [CONCENTRATION (M)]

FIG.18O

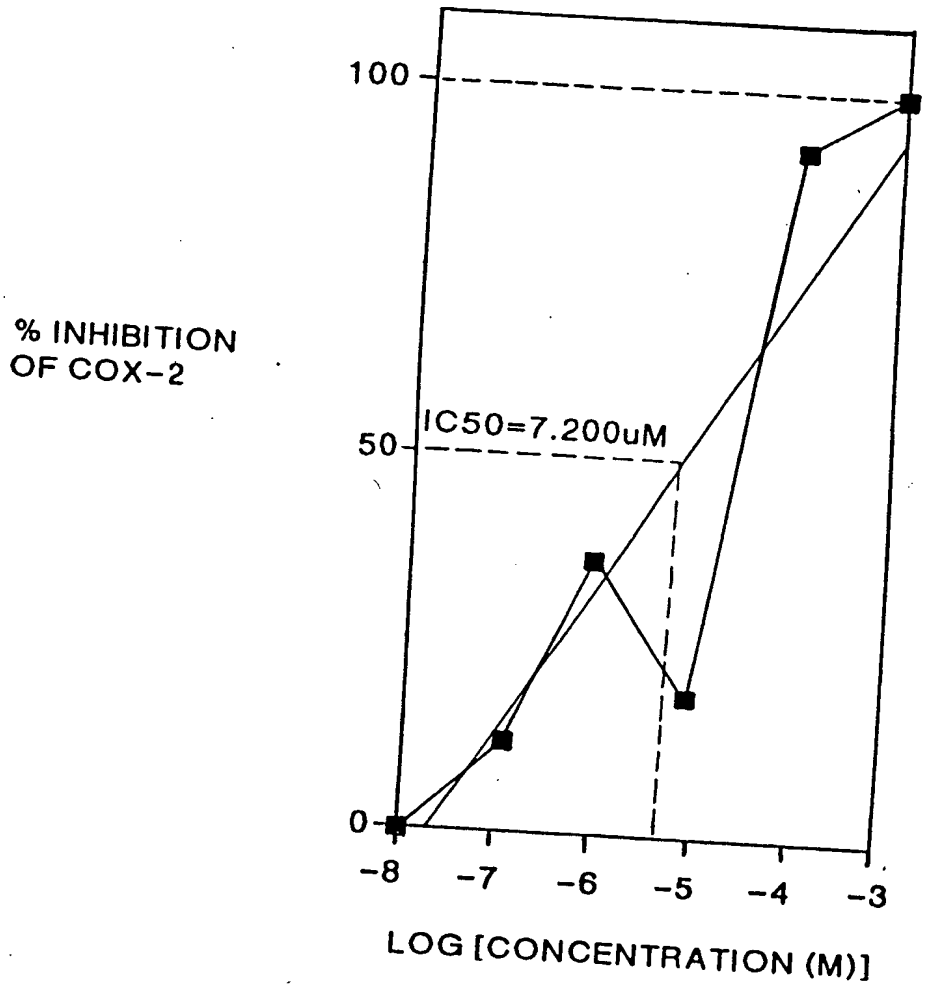

% INHIBITION
OF COX-2
100
50
0
IC50=7.200uM
-8
-7
-6
-5
-4
-3
LOG [CONCENTRATION (M)]

FIG.18P

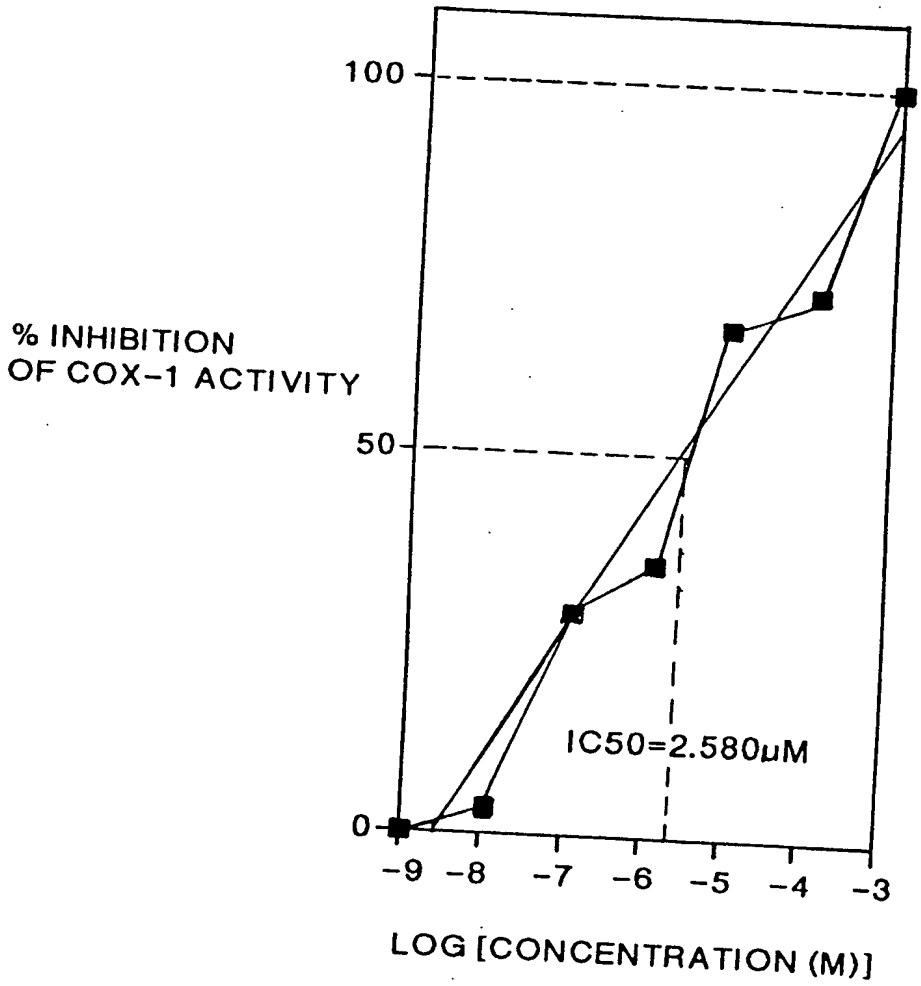

100
50
0
% INHIBITION
OF COX-1 ACTIVITY
IC50=2.580μM
-9
-8
-7
-6
-5
-4
-3
LOG [CONCENTRATION (M)]

FIG.18Q

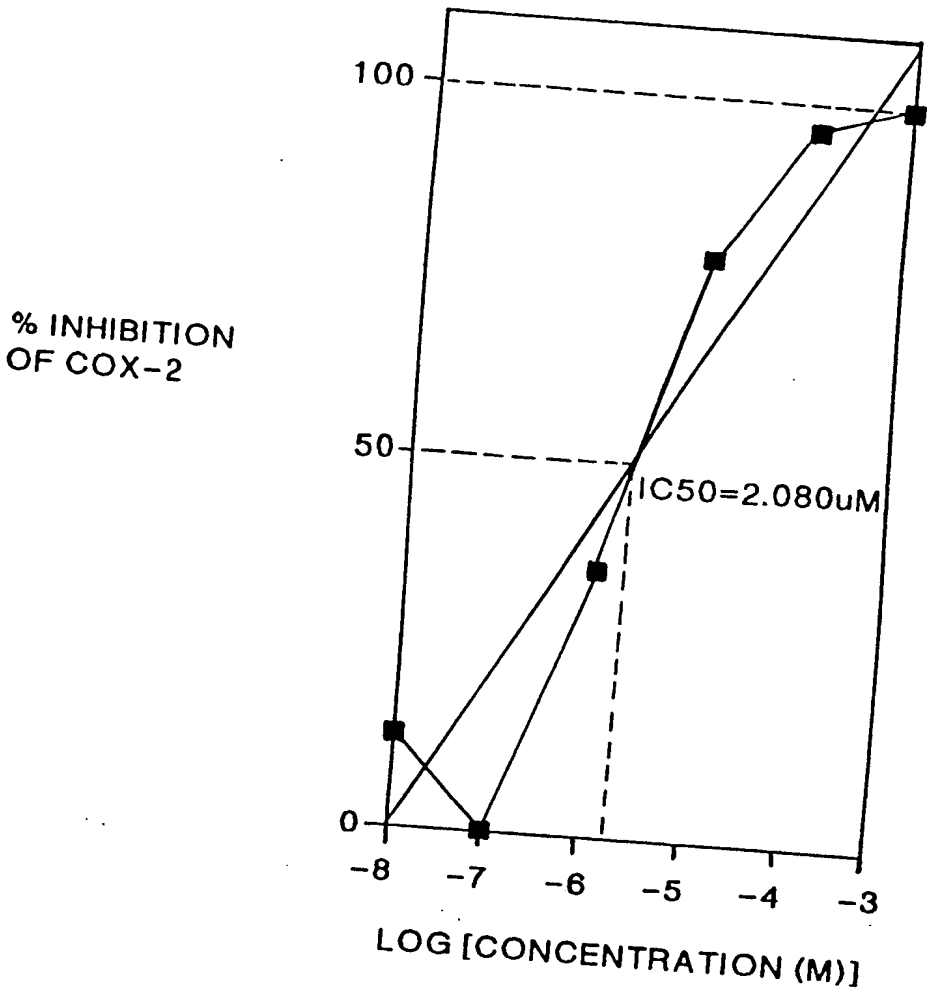

% INHIBITION
OF COX-2
100
50
0
IC50=2.080uM
-8
-7
-6
-5
-4
-3
LOG [CONCENTRATION (M)]

FIG.18R

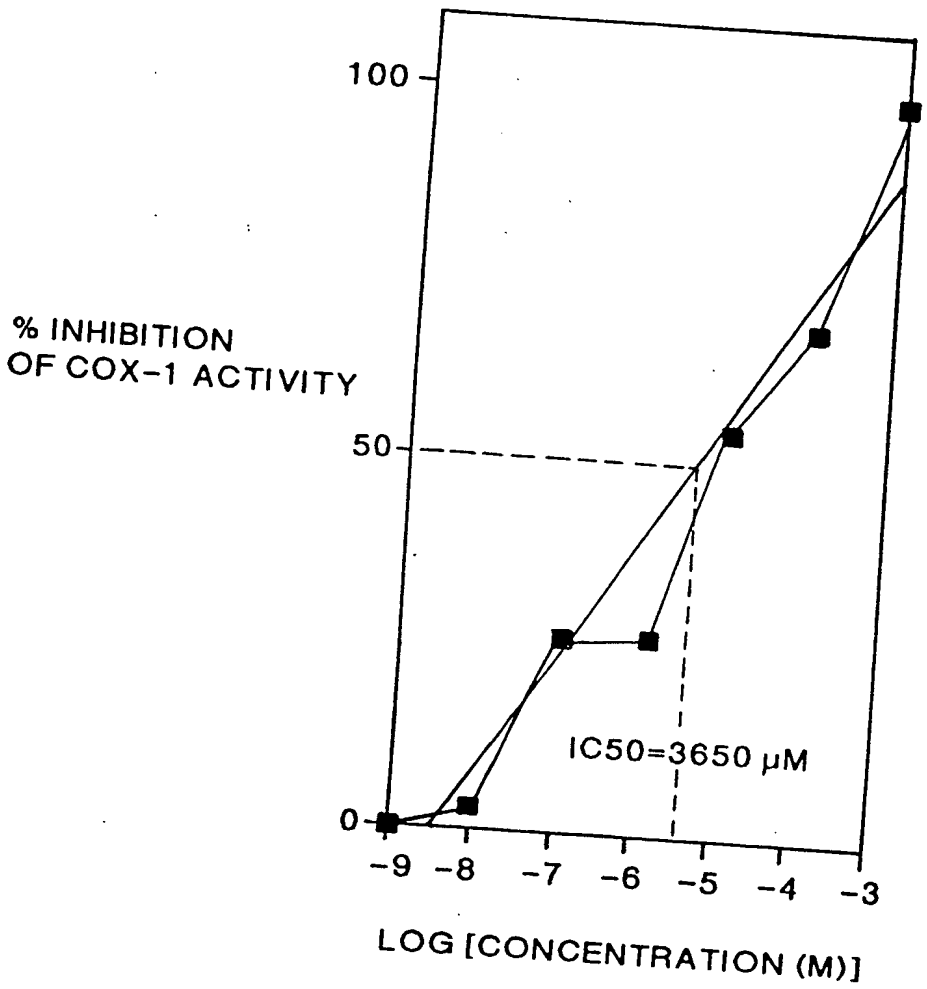

100
50
0
% INHIBITION
OF COX-1 ACTIVITY
IC50=3650 μM
-9 -8 -7 -6 -5 -4 -3
LOG [CONCENTRATION (M)]

FIG.18S

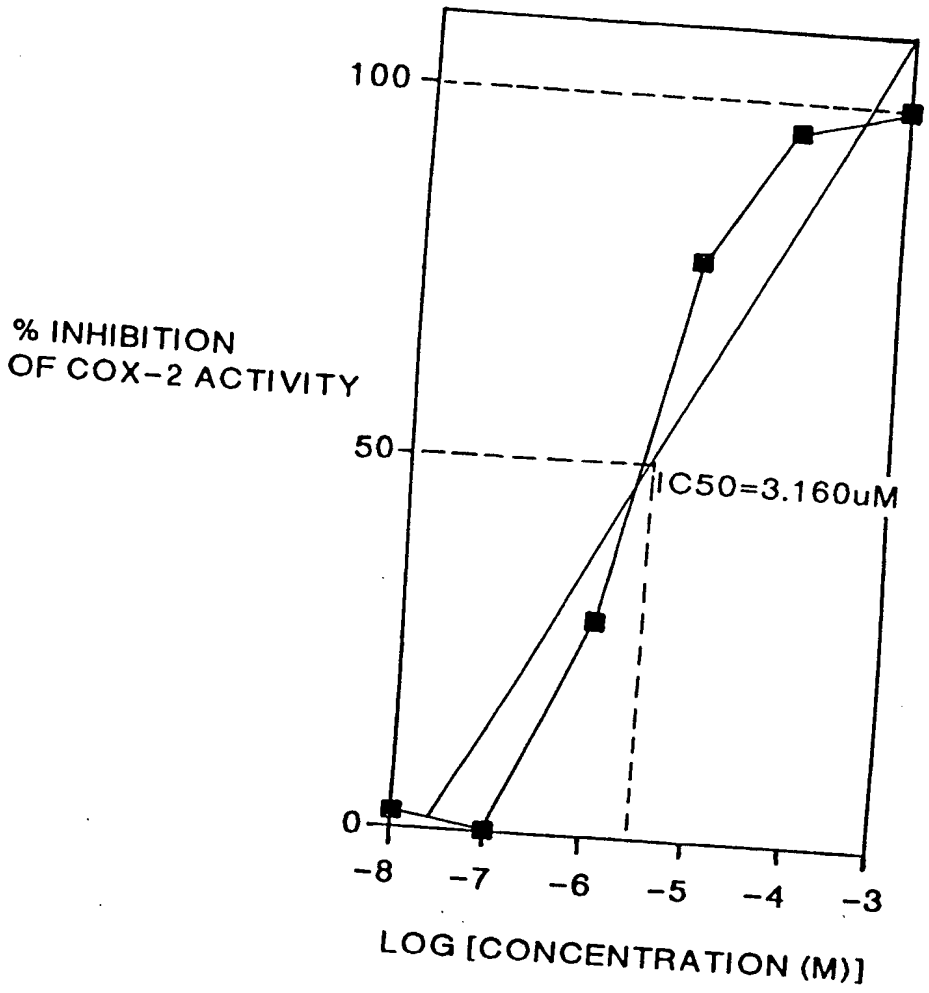

100
50
0
% INHIBITION
OF COX-2 ACTIVITY
IC50=3.160uM
-8
-7
-6
-5
-4
-3
LOG [CONCENTRATION (M)]

FIG.18T

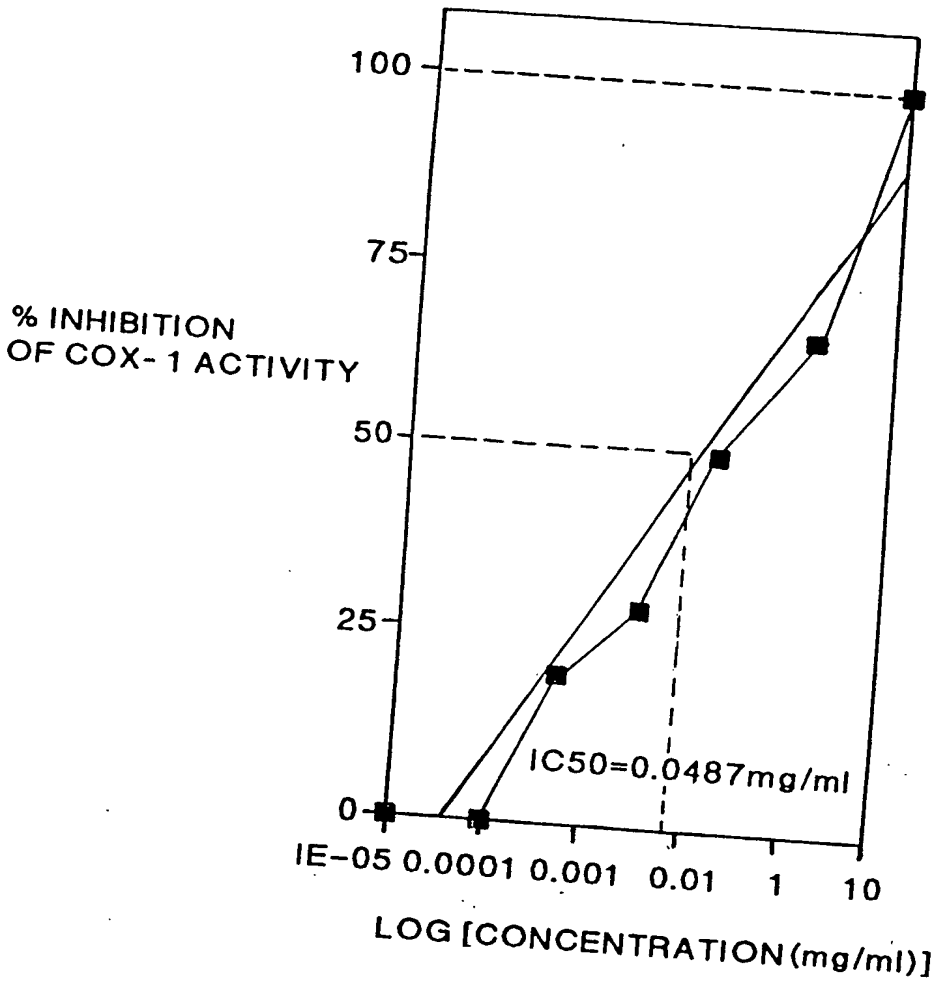

100
75
50
25
0
% INHIBITION
OF COX-1 ACTIVITY
IC50=0.0487mg/ml
IE-05 0.0001 0.001 0.01 1 10
LOG [CONCENTRATION (mg/ml)]

FIG.18U

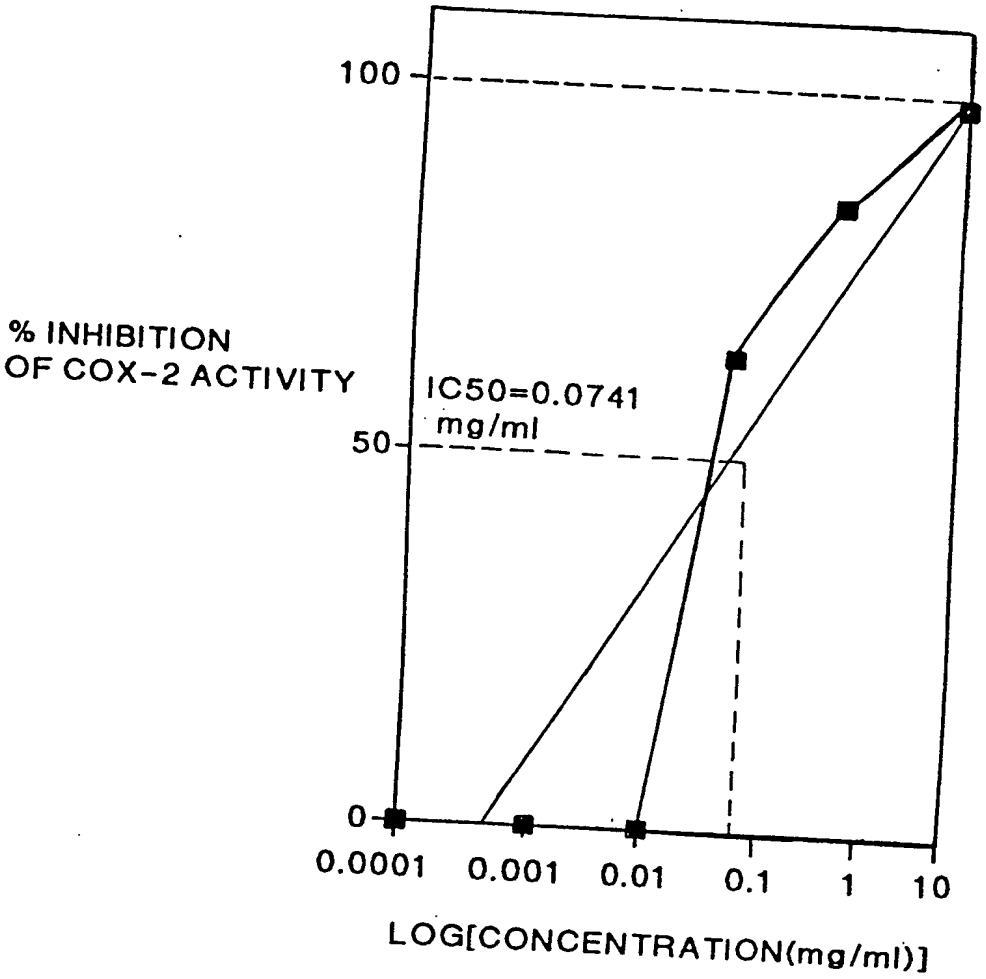

100
% INHIBITION
OF COX-2 ACTIVITY
IC50=0.0741
mg/ml
50
0
0.0001
0.001
0.01
0.1
1
10
LOG[CONCENTRATION(mg/ml)]

FIG.18V

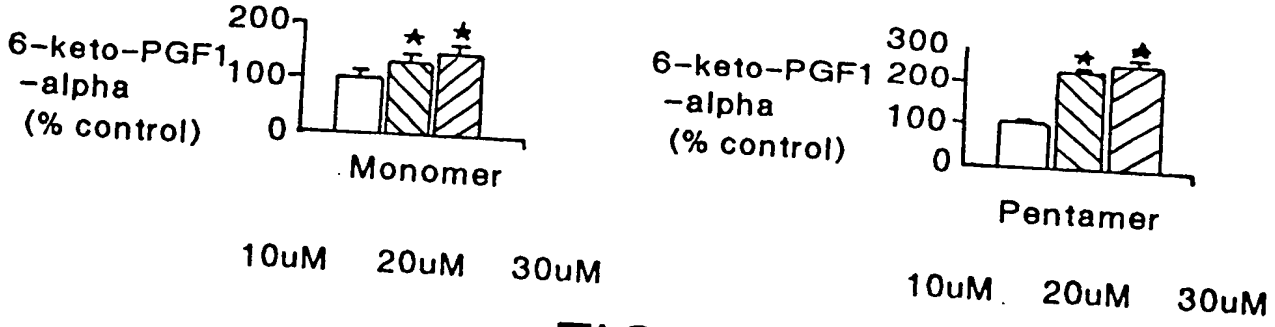


FIG.19A

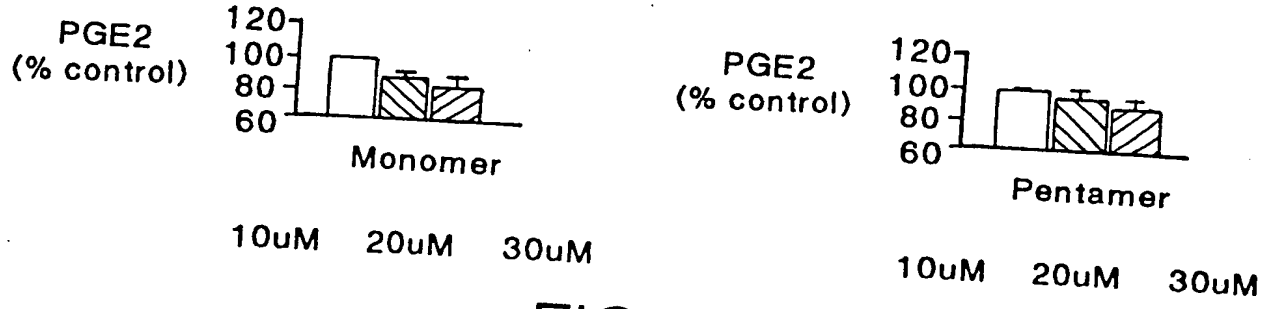


FIG.19B

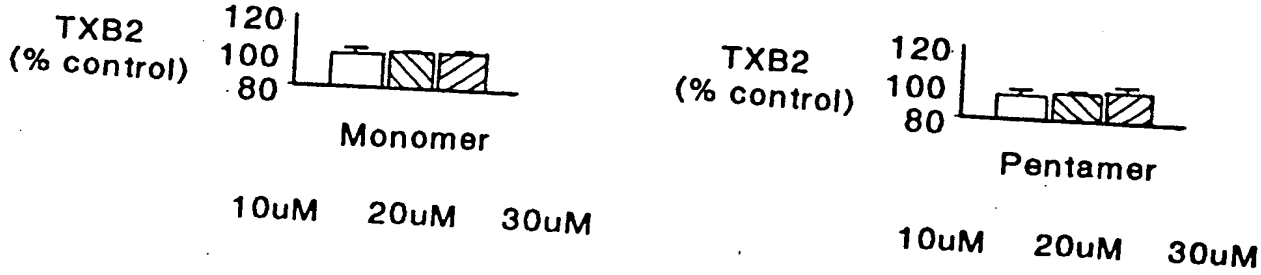


FIG.19C

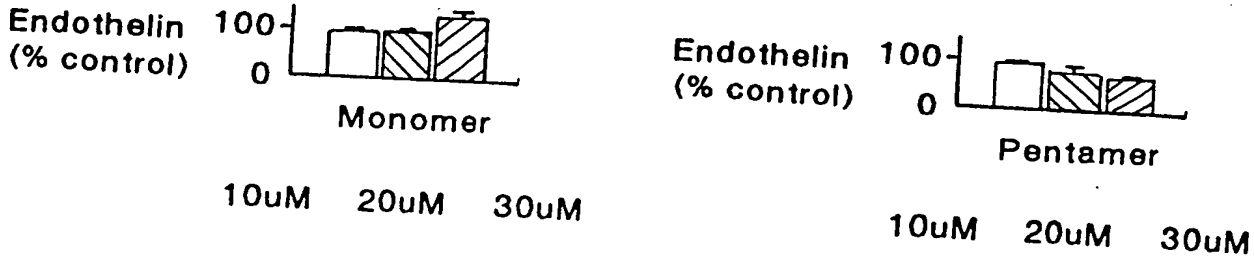


FIG.19D

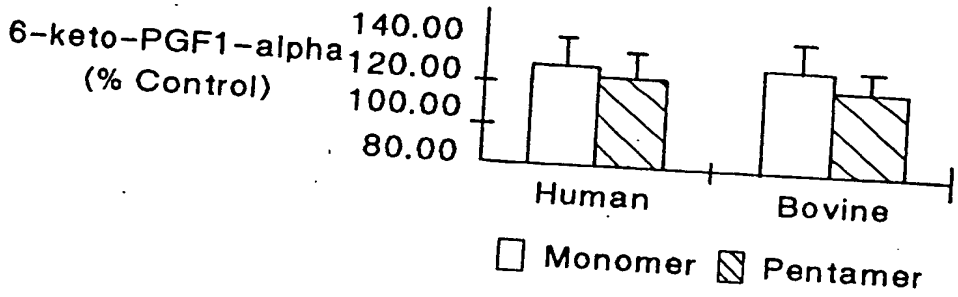


FIG.20A

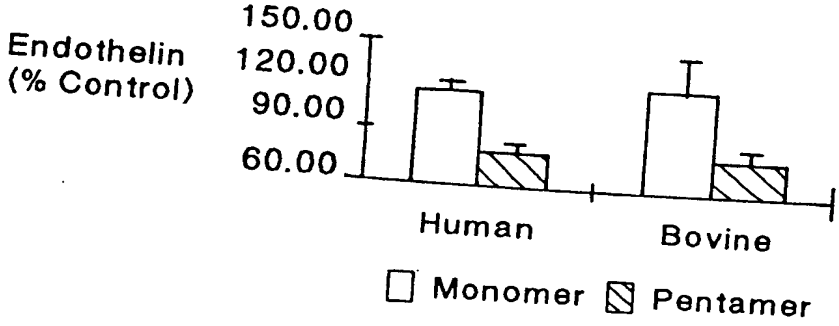


FIG.20B

FIG. 21

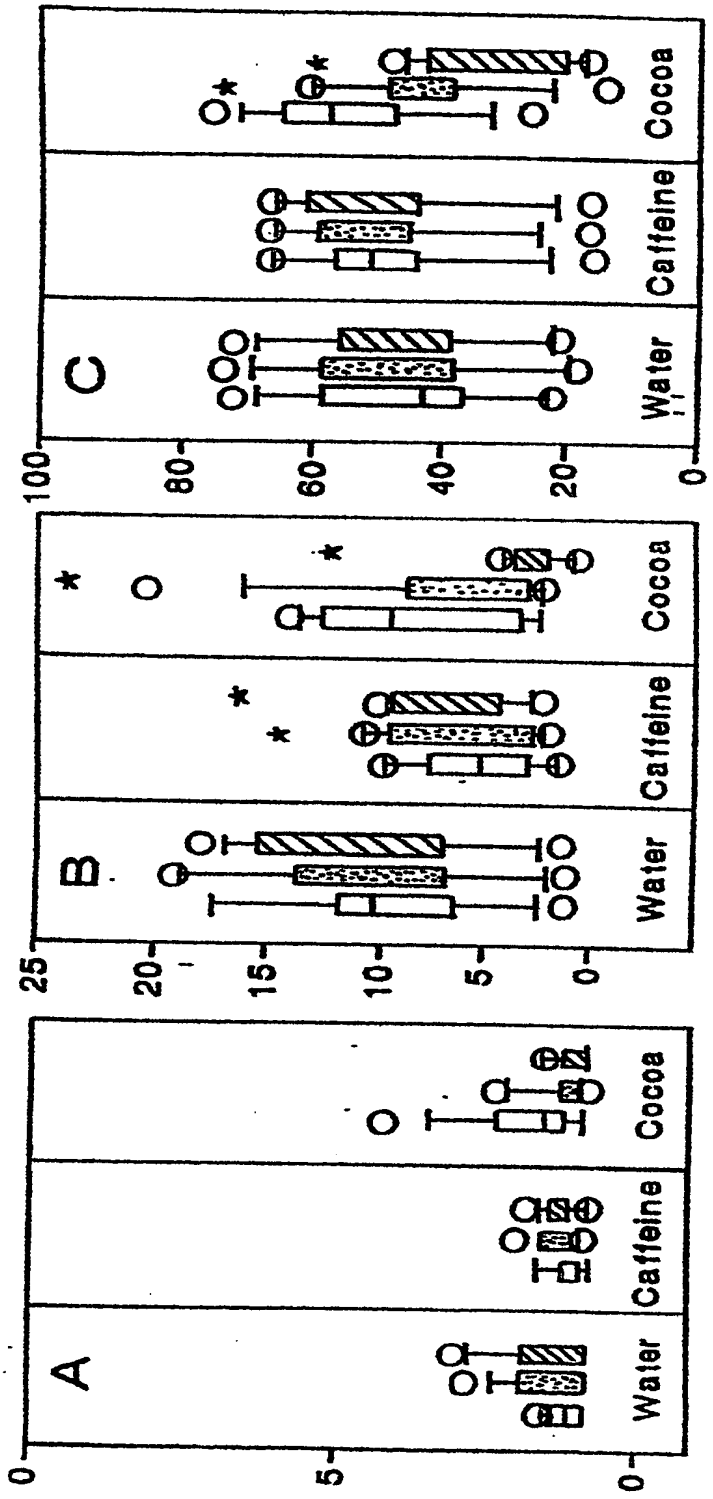
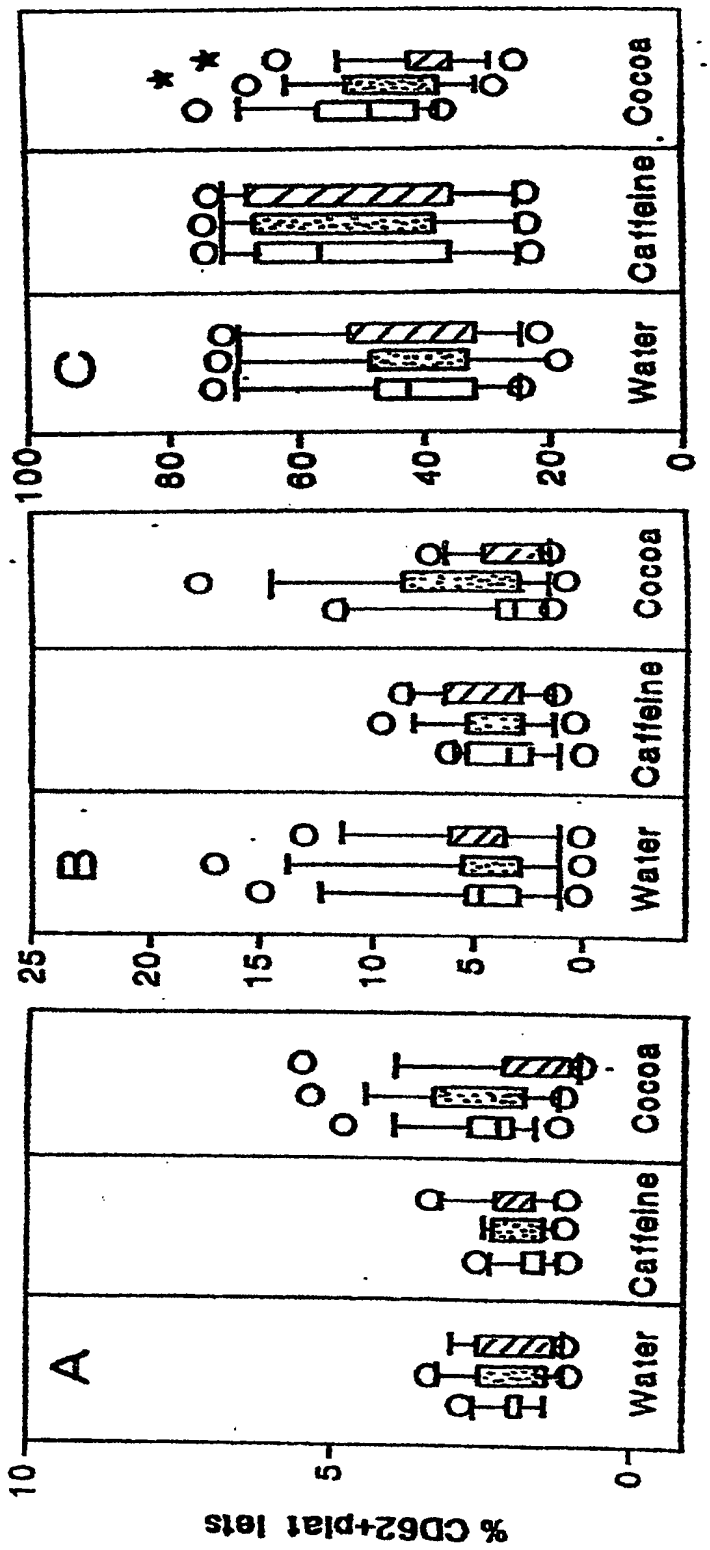

% PAC-1+ platelets
A
B
C
Water
Caffeine
Cocoa
0
5
0
25
20
15
10
5
0
100
80
60
40
20
0

FIG. 22


% CD62+plat lets
A
B
C
Water
Caffeine
Cocoa
0
5
10
15
20
25
40
60
80
100